Session Title: The Beauty of Water and Microorganisms
Session Start Date Time: 6/8/2018 11:00:00 AM
Session End Date Time: 6/8/2018 12:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 9026
Poster Board Number:

Abstract Title:
Bacterial and Viral Community Dynamics During Bloom Seasons in Lake Erie and An Inland Lake

Primary Author Block:
S. Lee, J. Lee, I. Mrdjen; The Ohio State Univ., Columbus, OH

Abstract Body:
Background: Anthropogenic nutrient loading and environmental changes increase the frequency and severity of cyanobacterial bloom events. Excessive growth of cyanobacteria and cyanotoxins have negative impacts on environmental and public health. However, the ecology of bloom dynamics, especially cyanobacteria and cyanophages, in lakes is poorly understood. The objective of this study is to understand the ecological dynamics of cyanobacteria and cyanophage communities, together with microcystin variations, during bloom seasons in western Lake Erie and Buckeye Lake, Ohio, USA.

Methods: Water samples were collected from both lakes between June and September 2016, and analyzed for water quality parameters, including nutrients, phycocyanin, chlorophyll-a, and microcystins. A metagenomic analysis was performed to determine bacterial and viral communities and their diversity. Total cyanobacteria and toxin producing cyanobacteria were also quantified with quantitative PCR. Results: The concentrations of chlorophyll-a and microcystins were higher than the WHO and USEPA eutrophication guidelines for recreational activities and drinking water. The results of bacterial community analysis at the phyla level show that Proteobacteria and Cyanobacteria were dominant in both lakes. However, the dominant genus within the cyanobacteria group was different: Microcystis dominated in Lake Erie and Planktothrix in Buckeye Lake. The qPCR analyses also confirmed that the level of the Microcystis toxin gene was higher than the Planktothrix toxin gene in Lake Erie while Buckeye Lake had more Planktothrix toxin genes than Microcystins toxin genes. In both lakes, the virus community was dominated by Podoviridae that have a large bacterial host range. Conclusions: This is the first study characterizing a cyanophage community in both lakes. These findings can enhance our understanding of their biology and characteristic complexes, and clarifies microbial interactions and structure in bloom-affected western Lake Erie and other inland lakes.
Abstract Title:
Straight from the Tap: A Real-Time Biofilm Model for Med. Devices Containing Non-Sterile Water

Primary Author Block:
D. Wolloschec, A. Garg, V. M. Hitchins, J. W. Weeks; US Food and Drug Admin., Silver Spring, MD

Abstract Body:
Background: Heater-Cooler Devices (HCDs) are important medical devices for patient thermoregulation during cardiothoracic surgeries. Manufacturer’s Instructions for Use (MIFU) recommend the use of sterilized water for reprocessing and filling of tanks. However, it is oftentimes common practice to use tap water to fill HCDs, leading to the development of biofilms inside these devices. Biofilms are known for their resistance to cleaning and disinfection, potentially leading to elevated bioburden inside HCD. Nontuberculous Mycobacteria (NTM) are emerging pathogens commonly found in tap water. Recently, M. chimaera and other NTMs have linked HCD biofilms to patient infections. Furthermore, the presence of NTMs is difficult to detect due to fast-growing contaminating organisms. Methods: Biofilms of NTMs were grown in sterilized tap water on stainless-steel coupons (SSC) to mimic the water tanks of HCDs. As per MIFU, water was changed weekly. After a month, biofilm was removed from the SSC and bacteria were enumerated. Dilute hydrogen peroxide was used to determine the effects of water treatment on biofilm formation. Additionally, chemicals were tested to remove fast-growing organisms without inhibiting NTM recovery. Finally, various chemicals were analyzed for their ability to reduce all bioburden after biofilms were established. Results: NTMs can form robust biofilms in tap water. In the presence of hydrogen peroxide, the biofilm associated NTM counts were significantly reduced (p <0.001). Cetylpyridinium chloride, a chemical regularly used for decontamination, also negatively affected NTM counts, suggesting it might be less suitable for quantitation of NTM in mixed populations. Conversely, sodium hydroxide based decontamination solutions showed only a marginal impact on NTM viability. When disinfectants were compared for their activity against tap water-grown NTM biofilm on SSC, sodium hypochlorite was found to be less effective than peracetic acid based solutions. Conclusions: NTMs are resilient organisms, that our work demonstrates it can form biofilms in tap water on SSC. In this study, we investigated the effects of chemicals used for water treatment, decontamination, and disinfection of NTM biofilms. Water treatment with hydrogen peroxide reduced bioburden on the SSC and this effect was dependent on the species. In addition, sodium hydroxide based solutions are more adequate decontaminants to quantify NTMs. Lastly, peracetic acid based solutions are more effective at reducing NTM bioburden from SSC.
Session Number: 27
Session Type: Poster Talk
Session Title: The Beauty of Water and Microorganisms
Session Start Date Time: 6/8/2018 11:00:00 AM
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Session Primary Track: Applied and Environmental Science
Abstract Control Number: 9483
Poster Board Number:

Abstract Title:
Microbiota of Surface Water Sourced from Dairy Farms in South Western Ontario

Primary Author Block:
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Abstract Body:
Background: Surface waters used in dairy operations play an important role in milk safety and the dairy cow’s health. The present study characterized the diversity of microbiota of untreated surface waters used in dairy farms. Method: Fifteen dairy farms, designated as A-O, were selected for bacteriological analysis. Water samples were collected biweekly from December 2015-March 2016 and then monthly from April 2016 to October 2016. Total bacteriological plate counts were performed by the standard membrane filtration method using Sorbitol MacConkey Agar (SMAC) for colony count, Chromocult Coliform Agar (CCA) and CCA + 4 μg/mL ceftiofur to screen resistant E. coli. For 16S rRNA gene sequencing and analysis, total DNA was extracted from surface waters filtered membranes of 14 farms sampled from August to December 2016. Results: Significant differences in colony forming unit per 100 ml (CFU/100ml) were detected between each dairy farm over the sampling period. The CFU counts on SMAC varied from 2.48 to 4.56 log10 CFU/100ml. E. coli was frequently isolated from water of farms B, F and I while Klebsiella spp. were more prevalent in the water from farms F, G H, and I in comparison to the other farms. The total taxonomic composition across all farms at the phylum level was dominated by Proteobacteria (34%), Cyanobacteria (21.8%), Bacteriodes (17.6%), Actinobacteria (17.3%) and Verrucomicrobia (5.5%), whereas Nitrospira, Spirochaetes, Thermi, Acidobacteria, Firmicutes, Gemmatimonadetes, Armatimonadetes, Chlorobi, Planctomycetes and Chloroflexi were in lower abundance (0.1 - 1%); however there was substantial variation between farms. Principal co-ordinate analysis (PCoA) of unweighted UniFrac distance matrices revealed clustering of the different collection time-points within the majority of farms, as well as a broader clustering of different time-points. The results indicate the presence of farm-specific surface water microbiota that underwent similar seasonal compositional changes. No significant differences in alpha-diversity (PD whole tree; p<0.05) were observed between any of the farms. Conclusion: Data showed diversity of bacterial communities in surface waters, both quantitatively and qualitatively on dairy farms in the Southwest Ontario that may help in developing efficient water treatment systems to preserve food safety and animal health.
Abstract Title:
The Impact of Riverine Infiltration on Groundwater Aquifer Microbial Communities
Primary Author Block:
N. J. Gayner, M. J. Salo, T. Grundl, R. J. Newton; Univ. of Wisconsin-Milwaukee, Milwaukee, WI
Abstract Body:
Shallow groundwater aquifers are an important agricultural, industrial and domestic drinking water source, and in the U.S. they account for 25% of all freshwater used. However, given their close connection to the surface, shallow groundwater aquifers are often altered by anthropogenic activities in the watershed. Microorganisms play a key role in groundwater biogeochemical reactions influencing water quality and the treatment processes needed to maintain potable water, but relatively little is known about how connections to surface water influence system biogeochemical stasis. In this study, three aquifer wells were examined within the same groundwater system located near the Fox River in Waukesha, WI. A significant portion of the Fox River’s flow comes from upstream wastewater treatment plant (WWTP) effluent. Our previous research indicated two wells are infiltrated by river water while the third well, approximately 1 mile away from the Fox River, is, seemingly, not infiltrated. There is no clear understanding of how or if river water infiltration will alter the microbial community, protein synthesis potential, and/or biogeochemistry of these drinking water wells. To address these questions, during the summer of 2017, water samples from the two contaminated wells and one pristine well were sampled on eight occasions. 2-3 L of water were filtered through a series of inline filters with decreasing pore-sizes (3 µm, 0.2 µm, and 0.1 µm). A preliminary analysis of a subset of samples from the 0.2 µm and 0.1 µm filters from all three wells using DAPI fluorescent stain and microscopy suggests microbial concentrations around 10^5 cells per mL of water in all three wells. 16S rRNA gene sequencing (illumina Mi-Seq) indicated the bacterial community composition of the wells differ greatly from those present in the WWTP effluent and Fox River and consist predominantly of unclassified taxa and bacterial guilds typical of groundwater, such as denitrifiers, iron-oxidizers, and sulfide-oxidizers. 16S rRNA gene sequencing on simultaneously extracted DNA and RNA from free-living groundwater microorganisms was used to characterize the microbial communities and protein synthesis potential of the communities (ribosomal RNA:DNA ratios) to identify patterns related to river water infiltration.
Session Title: The Beauty of Water and Microorganisms
Session Start Date Time: 6/8/2018 11:00:00 AM
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Session Primary Track: Applied and Environmental Science
Abstract Control Number: 9589
Poster Board Number:

Abstract Title:
Elucidating the Long-Term Impact of Disinfection Strategies on the Drinking Water Microbiome

Primary Author Block:
Z. Dai1, M. Sevillano2, S. T. Calus1, Q. Bautista de los Santos3, U. Z. Ijaz1, A. J. Pinto2; 1Univ. of Glasgow, Glasgow, United Kingdom, 2Northeastern Univ., Boston, MA, 3Univ. of Michigan, Ann Arbor, MI

Abstract Body:
Background: Disinfection is the common approach for controlling microbial growth in drinking water systems and is routine practice in USA, UK, etc. In contrast, some countries in western Europe, (e.g. Netherlands), provide consumers with drinking water free from any disinfectants (1, 2). The contrast in microbial control strategies between these systems provide an ideal opportunity to investigate long-term impacts of disinfection on the drinking water microbiome. In this study, we aim to utilize genome-level information to identify microbial metabolic traits under strong selection in both types of systems. We are particularly focused on candidate phyla radiation (CPR) (3), which are highly prevalent in drinking water systems and their characterization may provide insights into the minimal metabolic capacity required to survive in oligotrophic drinking water systems. Methods: Samples were collected from the 15 drinking water systems in Netherlands, England, Wales, and Scotland. A range of chemical parameters were analyzed for each sample. Microbed were harvested from the samples by filtering through 0.22 μm Sterivex filters. DNA was extracted directly from the filters, prepared for sequencing using Nextera XT kit, and sequenced on Illumina HiSeq 2500 to obtain 250-bp paired-end reads. Reads were quality trimmed (4) and assembled using MetaSPAdes (5). Coverage information was generated by mapping reads against the generated scaffolds longer than 500bp. Scaffolds longer than 2500bp were clustered into metagenome assembled genomes (MAGs) using CONCOCT(6), and the completeness of output results were validated by applying CheckM (). Anvi’o (8) was used curate MAGs manual curation and phylogenetic placement. Results and Conclusions: More than five million scaffolds longer than 500bp were generated which accounted for 67-99% of the all reads from each sampling location. Composition and coverage based binning resulted in nearly 700 MAGs, with 261 high quality MAGs. Of these, 140 and 121 MAGs are derived from system with and without a disinfectant residual. We are in the process of curating these MAGs which will be followed by determination of SAAV/SNV ratio for each MAG. This will be followed by determining the level of selective stress in system, and the metabolic pathways of organisms’ subject to high selective stress will be reconstructed. In addition, we will also identify core metabolic traits shared in both disinfected and non-disinfected systems and compare dispensable genomes which are unique to these different systems.
Abstract Title:
Occurrence and Antimicrobial Susceptibility of Vibrio parahaemolyticus Isolated from Oysters in Pacific Northwest of Mexico
Primary Author Block:
J. Velazquez-Roman1, L. Hernandez-Diaz1, N. Leon-Sicairos1, A. Guadron-Llanos1, H. FLORES-VILLAŞEÑOR1, S. Muro-Amador1, J. Vidal1, L. Calderon-Zamora1, E. Acosta-Smith1, U. Angulo-Zamudio1, G. Tapia-Pastrana2, J. Medina-Serrano1, M. Valdez-Flores1, A. Canizalez-Roman1; 1Centro de Investigación Aplicado a la Salud Públ. a Facultad de Med., Univ. Autonoma de Sinaloa, Culiacan, Mexico, 2Hosp. Regional de Alta Especialidad de Oaxaca, Oaxaca, Mexico, Oaxaca, Mexico
Abstract Body:
Background: Vibrio parahaemolyticus is an important cause of foodborne illness causing outbreaks and sporadic cases associated with the consumption of raw or partially cooked shellfish or mishandled marine products. Since 2004, pandemic V. parahaemolyticus strain O3:K6 has endemically established in the Pacific Coast of Mexico. Methods: The present study evaluated the serotypes by using a commercially available V. parahaemolyticus antiserum, distribution of virulence genes (tdh and trh) and presence of pandemic O3:K6 strains (tdh, toxRS/new, and orf8) by PCR, and antibiotic resistance of most commonly used were investigated in oyster samples from 2012 to 2014. Results: A total of 105 (n=33, 2012; n=35, 2013; and n=37, 2014) oyster samples were collected from Pacific coast and analyzed for the presence of V. parahaemolyticus. Of which 55.23% (58/105) belonged to V. parahaemolyticus, detecting 51.5%% (17/33), 57.1% (20/35) and 56.75% (21/37) in 2012, 2013 and 2014 respectively in oyster samples. Ten serovars were identified in serotyping, with the predominantly serotype OUT:KUT with 27.58% (16/58). Finding 2 new serotypes: O4:K13 and O8:K22, who were not previously reported in our investigations conducted in clinical, shrimp, seawater and sediment samples from 2004 to 2013. Toxigenic clones (tdh and/or trh positive) with 17.24% (10/58) were detected; but no pandemic (tdh, toxRS/new, and orf8) traits were detected. Among V. parahaemolyticus strains isolated, 86.2% showed resistance to at least one antibiotic, all the strains had resistance to ampicillin with 100% and high susceptibility was obtained from tetracycline (96.6%) and nalidixic acid (91.4%). Conclusion: We detected a high proportion of V. parahaemolyticus in oyster, with a high diversity of serotypes and antibiotic resistance to commonly therapy used. Thus, the presence of pathogenic strains in seafood such as oysters and antibiotic resistance are a matter of concern for public health, as the potential of outbreaks along of the northwest of Mexico is now well established. Continue monitoring of seafood and shellfish should be established in coastline of the Pacific Ocean northwest of Mexico.
Abstract Title:
Microbial Signatures as Trace Evidence in Residential Burglaries
Primary Author Block:
J. T. Hampton-Marcell1, G. Duncan2, J. V. Lopez2, J. A. Gilbert3; 1Univ. of Illinois at Chicago, Chicago, IL, 2Nova Southeastern Univ., Fort Lauderdale, FL, 3Univ. of Chicago, Chicago, IL
Abstract Body:
Background: Humans maintain a highly individualized microbial signature on their skin, and shed approximately 36 million bacterial cells per hour into their immediate environment, creating the possibility that the microbial signature of an individual could be reliably detected in an environment. The detection of individual microbial signatures in the built-environment has the potential to be utilized as another layer of trace evidence for forensic investigations. Methods: A series of mock burglaries for ten homes in Naperville, IL (n = 5) and Fort Lauderdale, IL (n =5) in August 2016 and March 2017. Various surfaces within the homes along with nares- and hand-associated samples from homeowners and mock burglars were sampled prior to and following entrance of burglars. Microbial community structure was analyzed using deblurred sub-OTUs from the 16S rRNA gene. Microbial assemblages unique to each individual were mapped from nares- and skin-associated samples, where unique assemblages from the burglars were mapped back to the various homes sampled. Studies analyzing longitudinal characterization of the human microbiome in the built-environment were combined with the burglary study to examine reproducibility. Results: Microbial community structure significantly explained variation among individuals for nares-associated (Adonis, p < 0.01, R = 0.817) and hand-associated (Adonis, p < 0.01, R = 0.666) microbiota. For the 400+ participants, more than 8000 unique microbial assemblages were recovered from nares- and hand-associated samples, where unique microbial combinations specific to an individual were observed across all studies. Unique microbial assemblages mapped burglars to homes they burglarized with an accuracy greater than 75%. Conclusion: This study demonstrates unique microbial assemblages are recoverable from human-associated samples and that these unique assemblages are left behind in immediate spaces humans interact with. Furthermore, profiles generated from the combination of unique microbial assemblages found among individuals to potentially serve as a forensic tool.
Abstract Title:
Quantitative Assessment of Agricultural Water Quality, Human and Animal Feces for Prevalence of Arcobacter Faecis And A. Lanthieri

Primary Author Block:
I. KHAN1, M. G. Miltenburg1, M. Cloutier1, O. Islam1, D. R. Lapen1, G. Wilkes1, G. Talbot2, E. TOPP3; 1AGRICULTURE AND AGRI-FOOD CANADA, OTTAWA, ON, Canada, 2AGRICULTURE AND AGRI-FOOD CANADA, Sherbrooke, QC, Canada, 3AGRICULTURE AND AGRI-FOOD CANADA, LONDON, ON, Canada

Abstract Body:
Background: Arcobacter faecis and A. lanthieri, isolated from human and animal fecal sources, are two newly classified members of genus Arcobacter. Since these species are closely related to A. butzleri, A. cryaerophilus and A. skirrowii that cause diseases in human and animals, this study investigated the prevalence of these species in surface water collected from a Canadian agricultural watershed located in South Nation River watershed eastern Ontario and fecal samples from human and animal sources by culture-independent direct DNA-based species-specific quantitative PCR assay. Methods: Initially, we developed and optimized species-specific quantitative real-time PCR assays based on the variable regions of rpoB and gyrA housekeeping genes for A. faecis and A. lanthieri, respectively. Specificity of primers and amplicons of each target reference strains were verified and confirmed by dissociation curve analysis using variable DNA concentrations. In order to demonstrate the utility of the approach in real environmental situations, the developed protocol was further validated by quantifying the prevalence of each species in environmental samples collected from water and various fecal sources. A total of 306 agricultural surface water samples, 19 human and 249 livestock, poultry and other domestic animal fecal samples were analyzed. Results: Overall, A. faecis was detected at the frequency of 14% (n= 42) in water with concentrations ranging from 2.5x103 to 4.9x105 cells/100 ml compared to fecal samples (7%; n= 19) where concentration ranges from 1.7x101 to 1.2x104 cells/g, respectively. However, A. lanthieri was detected at a low frequency in water (3%; n= 8) and fecal samples (4%; n= 11) with concentrations ranging from 1.8x104 to 4.1x105 cells/100 ml in water and 4.8x101 to 3.3x104 cells/g in fecal samples. Conclusions: This study provides a rapid tool for obtaining quantitative data on detection and routine testing of A. faecis and A. lanthieri in recreational, irrigation and drinking water and diagnostic laboratories.
Abstract Title:
Effect of Inorganic Mercury on the Abundance of Antibiotic Resistance Genes in Soil
Primary Author Block:
K. R. Mahbub1, N. Siboni1, J. R. Seymour1, M. Megharaj2, A. E. Franks3, M. Labbate1; 1Univ. of Technology Sydney, Sydney, Australia, 2The Univ. of Newcastle, Newcastle, Australia, 3La Trobe Univ., Melbourne, Australia
Abstract Body:
Background: Antibiotic resistance genes (ARGs) are commonly found in environments contaminated with human and animal waste. In these environments, microorganisms can interchange genetic traits through lateral gene transfer processes (LGT) allowing for spread of ARGs across bacterial communities and with the assistance of a variety of mobile genetic elements (MGEs) such as class 1 integrons, novel ARGs can be integrated into ARG loci. Recent studies have reported that heavy metals are able to co-select for ARGs (Ji et al. 2012). Therefore, it is important to investigate how the chemistry of heavy metals in soils influences the abundance of ARGs and whether removal of co-selection impacts on ARG abundance. Methods: In this study, two soils with different physicochemical properties and mercury (Hg) gradients were investigated to determine whether a dose-response correlation between Hg and ARGs was present. The soils contained <0.05 mg/kg (control soil) to approximately 250 mg/kg of spiked inorganic Hg. Different ARGs and MGEs namely intI, sull, tetA, tetB, dfrA1, vanB and qnrs were quantified as copy numbers per kg soil using qPCR technology (Berglund et al. 2014). The effect of Hg on the total microbial population was estimated by quantifying the dehydrogenase enzyme activity (DHA), which is a respiratory enzyme that is a proxy for the total active microbiota. The ARG copy numbers were then normalized by DHA to estimate the ratio of ARGs in the live microbial community. Results: All targeted ARGs and MGEs, except vanB were detected at various quantities in control soils, which increased at certain concentrations of Hg. In both soils, a dose-response correlation between Hg and ARGs was present. A significant increase of the copy numbers relative to DHA was evident in the soils containing 5, 10 and 50 mg/kg Hg compared with the control soils. The relative increase of these genes in the Hg spiked soils clearly demonstrates the correlation between Hg and the selection of bacteria containing ARGs. There was a decline in the relative ARG copy number in soils with more than 100 mg/kg Hg, which is the concentration that is known to kill the majority of the soil microbes as evidenced by DHA estimates. Conclusions: The evidence from the present study raises a concern of the selection of ARGs in Hg contaminated sites. Our further studies focus on precisely estimating the critical Hg levels that triggers soil ARGs co-selection and developing a bioremediation technique that acts to remove Hg pollution thus minimising co-selection for ARGs.
Abstract Title:
Supplemental Carbon and Energy Sources Stimulate the Amplification of Carbapenem-resistant Klebsiella pneumoniae (CRKP) in a Defined Sink Drain Biofilm Reactor Model

Primary Author Block:
M. L. Burgos-Garay1, C. Ganim1, T. Davy1, R. Donlan1, A. Mathers2, S. Kotay2; 1CDC, Atlanta, GA, 2Univ. of Virginia Hlth.System, Charlottesville, VA

Abstract Body:
Background: CRKP have been isolated from sink drain P-traps in healthcare facilities, but the mechanism for their colonization, persistence, and amplification is unclear. We hypothesized that liquid waste discarded into the sink drain could provide a source of nutrients for CRKP to colonize and amplify in the P-trap biofilms. The effect of the addition of a soft drink product (Soda), an intravenous fluid (5% dextrose in water, D5W), or a nutritional shake (Shake) on the colonization and amplification of two CRKP strains in a polymicrobial biofilm was investigated in an in vitro model system designed to mimic a P-trap. Methods: CDC Biofilm Reactors (CBRs) containing autoclaved municipal tap water (ATW) alone, or ATW supplemented with Soda (50% v/v), D5W (50% v/v) or Shake (10% v/v) were inoculated with cultures of Elizabethkingia sp., Stenotrophomonas maltophilia, Cupriavidus metallidurans, Methylobacterium fujisawaense, and Micrococcus luteus in equal proportion and operated under continuous flow conditions for 28 d at room temperature to grow biofilms on stainless steel coupons. Four times daily the CBRs were mixed and refilled with ATW and 2 ml hand soap. Biofilm heterotrophic plate counts (HPC) were quantified using R2A. 28-d biofilms were inoculated with CRKP KPC+ (ST258) or CRKP KPC+ (1016) and samples were collected at 7, 14, and 21 d after inoculation and enumerated on LES mENDO agar. Results: Biofilm HPC ranged from 6.59 - 6.86 log10 CFU/cm2 in un-amended ATW and were lower when supplemented with Soda, D5W, or Shake. ST258 and 1016 colonized biofilms in un-amended ATW and were detected 21 days after inoculation without amplification. Soda-amended ATW significantly reduced biofilms with CRKP 7, 14, and 21 d after inoculation. Compared to ATW, the 21-d counts decreased for ST258 from 3.01 to 0.89 log10 CFU/cm2 (p=0.0004) and for 1016 2.47 to 0.94 log10 CFU/cm2 (p=0.0004). ST258 and 1016 counts increased in D5W-amended ATW and were significantly amplified at 14 d compared to un-amended ATW; for ST258, 2.97 to 3.84 log10 CFU/cm2 (p=0.002) and 2.87 to 3.73 log10 CFU/cm2 (p=0.006) for 1016. Addition of Shake significantly increased 14 and 21-d ST258 and 1016 biofilm counts. Compared to ATW, 21-d counts significantly amplified for ST258 (3.01 to 4.80 log CFU/cm2 (p=0.001), and 1016 (2.47 to 4.75 log CFU/cm2 (p=0.0004) in Shake-amended ATW. Conclusions: Healthcare personnel should avoid discarding liquid waste into handwashing sinks as this may provide a source of nutrients for CRKP, resulting in their amplification in sink drain P-traps.
Abstract Title:
Production of A Universal Plant-Based Substrate System for Cellulase Activity Assays

Primary Author Block:
k. Hefferon; Cornell Univ., Trumansburg, NY

Abstract Body:
Cellulases and other cell wall degrading enzymes are currently being engineered with improved traits for application in the breakdown of lignocellulosic biomass. The majority of assays with these ‘designer’ enzymes have been carried out using synthetic substrates such as crystalline bacterial microcellulose (BMCC). The use of synthetic substrates may not reflect the actual action of these cellulases on real plants. In the following study, suspension cell walls from several species of plant were examined as a possible alternative for synthetic cellulose substrates. Cell walls prepared from tobacco, rice and canola plant suspension cell cultures were found to work consistently and reliably as more realistic substrates than BMCC for cellulase degradation. Cell walls prepared from these suspension cultures could be preserved in a frozen state without a negative impact on cellulase activity. Mesophyll cells derived from shoot cultures from a variety of food crops, such as sugar cane, corn, potato and tomato were also examined as substrates for reproducibility in cellulase assays. The results of this study indicate that a new universal substrate based on plant cell wall tissue can conceivably be utilized to provide a more accurate representation of cellulase activity of plant biomass. These results can benefit both academic and industrial researchers alike with respect to the study of cellulase evolution, function and diversity, as well as for applications in biofuel development.
Isolation and Characterization of Phthalate-Degrading Bacteria from the Asian Carp Microbiomes and Riverine Sediments

Primary Author Block:
S. A. Kolb1, E. J. O’Loughlin2, T. C. Gsell1; 1Governors State Univ., University Park, IL, 2Argonne Natl. Lab., Lemont, IL

Abstract Body:
Phthalates have become ubiquitous in aquatic environments (Liang et al. 2008). The U.S. EPA has classified dimethyl phthalate (DMP), diethyl phthalate (DEP), and dibutyl phthalate (DBP) as top priority pollutants. Many phthalate-degrading bacteria have been isolated from various environments; however, few studies demonstrate the isolation of phthalate-degrading bacteria from aquatic organisms. Invasive Asian carp residing in contaminated environments absorb phthalates and ingest organic pollutants. The purpose of this study was to isolate phthalate-degrading bacteria and characterize the degradation kinetics of DMP, DEP, and DBP. We have identified efficient phthalate-degrading bacteria from the microbiomes of both Asian Carp species, the scale biofilm of silver carp, and riverine sediments. Sediment samples were obtained from the Calumet River located in Chicago, IL. Asian carp were collected from the Illinois River in Morris, IL. The gill rakers, scale biofilms, and fecal material from both Asian carp species in addition to sediments were enriched in minimum salt medium amended with a mixture of DMP, DEP, and DBP. DNA was extracted in the final enrichment for 16S rRNA amplicon sequencing. Bacteria isolated from enrichments were tested for their ability to grow on DMP, DEP, and DBP as sole carbon sources. Optical density measurements (OD600) and HPLC analysis were employed to characterize degradation kinetics. This study has revealed Asian carp can act as a source for isolating phthalate-degrading bacteria. Sediment and silver carp microbiome inoculated enrichments contained similar genera, but sediments contained a larger distribution of dominant genera than silver carp (Fig. 4). The bacteria isolated from the microbiomes of both Asian carp species were efficient in metabolizing phthalates as bacteria isolated from riverine sediments. All the bacteria isolated from this study may be useful for the bioremediation of phthalate compounds.
Abstract Title:
Lignocellulosic Material Enhanced Peroxidase Production by Ensifer Adhaerens Under Solid State Fermentation

Primary Author Block:
U. Nwodo, Male, Ayodeji Falade, Leonard Mabinya and Anthony Okoh; Univ. of Fort Hare, Alice, South Africa

Abstract Body:
Background: The increased industrial application potentials of peroxidase have led to high market demand which have outweighed the commercially available peroxidases. Consequently, the need for alternative and efficient peroxidase-producers is imperative. Hence, an investigation of peroxidase production potential of Ensifer adhaerens NWODO-2 (accession number KX640918) was undertaken in this study. Methods: The peroxidase production potential of Ensifer adhaerens NWODO-2 (accession number KX640918) was investigated the by screening for the presence of peroxidase genes using polymerase chain reaction (PCR); and the process conditions for peroxidase production in both submerged and solid-state fermentation systems were determined. Results: Peroxidase production by Ensifer adhaerens NWODO-2 was optimum at 48 h incubation with specific productivity of 12.76 U mg⁻¹ at pH 7, 30°C and 100 rpm in a kraft lignin fermentation medium supplemented with guaiacol as the most effective inducer and ammonium sulphate as the best inorganic nitrogen source. Upon valorisation of lignocellulosic materials as carbon source for the enzyme production, sawdust gave the best peroxidase yield (37.50 U mg⁻¹) under solid-state fermentation. BLAST search of the PCR-amplified peroxidase gene in UniProtKB showed 70.5% similarity to an uncharacterized protein in Ensifer adhaerens but phylogenetic analysis suggests that the gene might encode a catalase-peroxidase with an estimated molecular weight of 31.145 kDa and isoelectric point of 11.47. The nucleotide sequence of the detected gene was deposited in the GenBank under the accession number MF374336. Conclusions: The ability of Ensifer adhaerens NWODO-2 to utilize lignocellulosic materials for peroxidase production augurs well for industrial application as this would greatly reduce cost; which is a major challenge in industrial enzyme production.
Session Title: Investigating the Susceptibility of Exaiptasia pallida to Black Band Disease of Corals
Primary Author Block: P. Waikel, M. Rodriguez-Lanetty; Florida Intl. Univ., Miami, FL
Abstract Body:
Black Band Disease (BBD) is a polymicrobial, mat forming disease, affecting more than 64 species of reef building coral worldwide. It presents as a dark band separating denuded coral skeleton from apparently healthy tissue and can migrate at rates up to 1 cm per day, quickly devastating entire coral colonies. Virulence of this disease has been shown to increase at elevated temperatures. Traditional infection studies require field collection of coral and subsequent maintenance in aquaria, often including lengthy acclimation periods prior to the initiation of a study. The use of a model system to study BBD would mitigate these obstacles. The tropical sea anemone Exaiptasia pallida is well established as a model system for studying cnidarian-dinoflagellate symbiosis (including that of corals) and has gained momentum in recent years as a model for studying coral disease. E. pallida has been successfully infected with opportunistic coral pathogens including Vibrio spp. and Serratia marcescens. To assess its potential as a model system for this polymicrobial disease, we examined the susceptibility of E. pallida to BBD. To accomplish this, clonal CC7 anemones were challenged with BBD collected from an infected coral colony of Montastraea cavernosa. A total of 36 anemones were utilized across three temperatures (25°, 27°, and 32° C), with 6 control anemones and 6 BBD-infected anemones at each temperature. Within 24 hrs, behavioral response to infection was observed including tentacle retraction, locomotion, detachment from substrate, and ejection of acontia. Mortality was first observed at 48 hrs in the 32° treatment and by 6 days post-infection, 100% mortality of infected anemones was observed across all temperatures while controls remained alive and apparently healthy. A Kaplan-Meier survival plot was generated, followed by a log rank test in SPSS revealing a significant difference (p = .033) in survivability across temperatures. Further pairwise testing indicated a significant difference (p = .016) between the 27° and 32° treatments. Our results indicate that E. pallida is susceptible to BBD infection and that temperature may modulate susceptibility, providing preliminary support for the potential use of E. pallida as a model system for studying BBD. This would remove the necessity for field collection and difficult maintenance of corals in aquaria, making the study of this polymicrobial disease more accessible to researchers, promoting advances in understanding the etiology of this devastating coral disease.
Abstract:
Transcriptomics Reveal Novel Genes in Bifidobacterium longum ssp. infantis Bi-26 Involved in Metabolism of Human Milk Oligosaccharide 2'-fucosyllactose
Primary Author Block:
B. Zabel; DuPont Nutrition and Hlth., Madison, WI
Abstract Body:
Human milk contains essential nutrients required for infant growth, development, and protection from diseases while their own immune system and microbiota matures. Non-digestible complex oligosaccharides, human milk oligosaccharides (HMOs), are the third most abundant component of human milk. They reach the colon in an intact form and are subsequently fermented by the infant gut microbiota. HMOs can function as prebiotics for beneficial bacteria, often dominated by bifidobacteria, and shape the typical intestinal microbiota of the breast-fed infant. Research into how specific Bifidobacterium strains utilize individual HMOs and the metabolites they produce is limited, therefore we analyzed how Bifidobacterium longum ssp. infantis strain Bi-26 (DGCC11473) metabolizes 2'-fucosyllactose (2'-FL) and lactose in a medium as a sole carbon source. Transcriptomic data was obtained by growing B. longum ssp. infantis Bi-26 in modified Bifidobacterium Media 58 (DSMZ) containing 1% 2'-FL or lactose. Samples were taken at early phase (A600 0.25), at mid log (A600 0.5-0.7) and at late log (A600 0.9-1.1), after RNA isolation and treatment, samples were sequenced via HiSeq®2500 (Illumina) with a read length of 76bp (paired ends) with analysis being performed with DNASTAR®. Transcriptomic analysis revealed many novel gene clusters in Bi-26 that were involved in the breakdown/transport of 2'-FL that were identified to be up regulated (> two-fold change), and also shown to be statistically different than the lactose control (p-value <0.05). Individual genes identified were involved in the breakdown of 2'-FL as a whole, along with the breakdown of the constituent monomers; glucose, galactose and fucose. Three transport gene clusters were also shown to be up regulated (100-500 fold) that did not compare to previous B. infantis studies. We concluded that 2'-FL metabolism is a complex process involving many gene clusters throughout the genome compared to lactose which requires only a limited number of genes. These results provide valuable insight on the mode-of-action of 2'-FL utilization by Bi-26 in the developing gut.
Abstract Title:
Multidrug Resistant Salmonella enterica Subsp. Enterica Serovars Typhi and Koessen in Water and Wastewater in Ibadan Metropolis, Nigeria

Primary Author Block:

Abstract Body:
Background: Multidrug resistant Salmonella in water and wastewater are potential sources of human enteric infections. Increasing resistance of Salmonella enterica to commonly used antibiotics has become a matter of global concern. This study therefore assessed the risk of multidrug resistant Salmonella in industrial wastewater, rivers receiving industrial and domestic waste, potable water sources in close proximity. Methods: Two major rivers in Ibadan, southwestern Nigeria, Ona River (urbanization and industrial waste impact) and Ogunpa River (refuse dumping site of the inner city residents) were purposively selected for this study. Sanitary survey and microbiological analyses of water and wastewater were performed using standard methods. Antibiotic resistance profile of identified S. enterica isolates was carried out and multidrug resistant strains were identified using 16S rRNA gene sequencing. Results: Salmonella enterica serovars Typhi and Koessen were isolated from hand-dug well, borehole, packaged sachet water irrespective of high or low risk scores from sanitary survey as well as industrial waste, river receiving domestic waste. Isolates were resistant to Carbapenems, Macrolides, Folate pathway inhibitors, Phenicol, Cephalosporin III, Aminopenicillin, and Fluoroquinolone classes of antibiotics. Conclusions: This study has shown that sanitary risk score cannot be used to predict the presence/absence of Salmonella in water and wastewater. In addition, multidrug resistance in Salmonella enterica is on the increase and this is the first report of serovar Koessen in Nigeria.
Abstract Title:
Presence and Distribution of Carbapenem-Resistant Gram-Negative Bacilli in One Municipal Wastewater Treatment Plants of Colombia

Primary Author Block:

Abstract Body:
Background: The wastewater treatment plants (WWTP) are considered one important reservoir of bacterial resistance. Carbapenem-resistant Gram-negative Bacilli have been described in different WWTP in the world, however, few studies describe the distribution of these microorganisms in different points of WWTP. The objective of this work is to determine the presence of Carbapenem-resistant Gram-negative Bacilli along WWTP in Colombia. Methods: A cross-sectional study was conducted in one WWTP in Medellin. Once a month from January to July 2017, water samples were taken from 4 points along WWTP, influent, aerated tank, recycled sludge and effluent. Isolation of bacteria was performed on chromID® CARBA medium. Bacteria were identified by 16S rRNA and susceptibility testing was performed using the VITEK®-2. Molecular analyzes included PCR for detection of blaKPC, blaNDM and blaOXA-48 genes. Results: A total of 360 Gram-negative bacilli were isolated on ChromID carba plates (90 isolates for point). The carbapenem resistant bacteria detected were Aeromonas ssp (n = 58, 41%), followed by Enterobacter ssp (n = 38, 27%), Raoultella sp. (n = 12, 8%), Klebsiella ssp (n = 11, 8%), Citrobacter freundii (n = 8, 6%), Pantoea sp. (n = 7, 5%), Kluyvera spp. (n = 4, 3%), Escherichia coli. (n = 3, 2%) and Pseudomonas sp. (n = 1, 1%). High minimum inhibitory concentration (MIC) values of carbapenem were observed in these bacteria and their resistance pattern showed resistances for two or more tested antibiotics The carbapenemase KPC was detected in 142 isolates resistant to carbapenem and was more frequent in isolates of influent (n = 44, 49%) and effluent (n = 41, 44%) and less frequently in the recycled sludge (33%, n = 30) and the aerated tank (31%, n = 28). No other carbapenemases were detected. In general, the bacteria harboring KPC were detected in different sampling sites, but Aeromonas spp. were more frequently detected in recycled sludge. Conclusions: This study demonstrates the high prevalence carbapenem-resistant Gram-negative bacilli in different sampling sites of one WWTP, this shows the risk of dissemination of multidrug-resistant genes between environmental and opportunistic pathogens.
Abstract:
Multidrug-Resistant Toxigenic O1 El Tor and Non-O1/Non-O139 Vibrio Cholerae Strains In Water Sources of Communities Affected with 2013 Cholera Outbreak in Abeokuta, Nigeria

Primary Author Block:

Abstract Body:
Background: The aquatic environment serves as an important reservoir for Vibrio cholerae. Investigations of recurrent cholera outbreaks in Abeokuta centered more on clinical isolates of Vibrio cholerae. There is dearth of information on the circulating strains of toxigenic V. cholerae in water sources of communities that were affected in the 2013 cholera outbreak in Abeokuta as well as their post-epidemic risk mapping. Methods: Forty (40) water samples collected from geo-referenced taps, wells, borehole, pool, rocks and rivers were analyzed for V. cholerae using conventional methods as well as antibiotic selection technique (AST) for multidrug-resistant epidemic prone strains. Risk maps were generated using coordinates obtained from the sampling points and the total counts of presumptive Vibrio. Suspected Vibrio cholerae were further characterized using amplicons of sodB, ctxA and 16S rRNA genes. Results: Of the 40 water samples analyzed, 87.5% were found to be contaminated with V. cholerae ranging from 2.85 - 5.52 log10 CFU/mL. The risk map showed some areas to be in very high risk of epidemic prone V. cholerae. Four isolates were ctxA positive, 2 of which were selected by AST. Strains were identified as multidrug resistant atypical O1 El Tor (n=1) and non-O1/non-O139 (n=3) serotypes and were obtained from river and well water respectively. Phylogenetic analysis showed that the isolates were local strains and different from genebank deposited strains. Conclusions: Strains of Vibrio cholerae atypical O1 El Tor and non-O1/non-O139 including antibiotics-resistant strains constitute epidemic threat and risk in outbreak affected communities in Abeokuta, Nigeria.
Session Title: AES03 - Antimicrobial Resistance in the Environment: Revealing the Resistome
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Session Primary Track: Applied and Environmental Science
Abstract Control Number: 6083
Poster Board Number: FRIDAY - 778

Abstract Title:
Phylogenetic, Virulence Factors and Antimicrobial Resistance Profile of Escherichia coli Isolated from Calves with and Without Diarrhea

Primary Author Block:
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Abstract Body:
Escherichia coli comprise a group of bacteria with a huge genetic diversity what make them able to colonize different niches and to exit as a component of intestinal microbiota of animals and human but also capable to causing intestinal and extra-intestinal infections. Curli and cellulose are the major components of the E. coli extracellular matrix, and are highly associated with the colonization of biotic and abiotic surfaces. Curli and cellulose are highly prevalent in human intestinal isolates, but in relation to calf isolates study are scarce. To address the importance of these components to calf isolates genes csgA (curli) and bcsA (cellulose) were searched in this study. It is also known that diarrhea may cause a serious disturb in the intestinal homeostasis with changes in the intestinal microbiota. To evaluate this changes, we characterized sixty fecal E. coli isolates from calves (30 to 60 days old) with and without diarrhea according to the new Clermont’s phylogenetic classification; the presence of curli, cellulose and fimbriae (F4, F5, F6, F18, F4), as well, the antimicrobial susceptibility profile. Disk diffusion technique and PCR were used as methodologies. E. coli isolates from calves without diarrhea were classified as B1 (83.3%, 25/30), E (10%; 3/30) and unknown (6.7%; 2/30); whereas the isolates recovered from calves with diarrhea were classified as B1 (70%, 21/30), B2 (3.33%), C (3.33%, 1/30), D (3.33%, 1/30), E (13.33%, 4/30) and unknown (6.7%; 2/30). No one isolate from calves without diarrhea were characterized as multiresistant, but the diarrheic group presented 16 (53.3%) multiresistant isolates. Only isolates (30% - 9/30) from diarrheic calves were positive for fimbriae, being 16.7% (5/30) for F5 and 13.3% (4/30) for F18. Despite the diarrhea condition of the calves, the phylum group B1 remains the main phylogenetic group for both commensal and diarrheic-associated isolates; curli (csgA) and cellulose (bcsA) were highly conserved showing the importance of these components for E. coli from bovine intestinal origin and finally, E. coli isolates from animals with diarrhea showed a much higher resistance profile with emphasis to tetracycline, ampicillin, streptomycin and sulfonamide, following a worldwide trend.
Abstract Title:
Non-Culture Based Approach for Taxonomic and Resistome Profiling of Kitfo, An Ethiopian Beef Steak Tartar
Primary Author Block:
B. B. Eshetea1, N. Addy2, L. Ewing2, J. J. G. Beaubrun2, B. Eribo1; 1Howard Univeristy, Washington, DC, DC, 2FDA, Laurel, MD
Abstract Body:
Background: Kitfo is a version of beef steak tartar widely consumed in the Ethiopian community. It is made from raw minced beef mixed with clarified butter ghee and a blend multiple spice powders. Several studies using culture method have reported ‘Kitfo’ to be a potential source of various foodborne pathogens including Salmonella. Knowledge gap exists regarding the quality, safety and microbial profile of this product. Methods: The present study employs metagenomic shotgun sequencing to determine the microbiome and antibiotic resistance profile of Kitfo. Twenty-five grams of retail samples were homogenized in 225ml of trypticase soy broth and the homogenate was incubated at 37°C for 18-24h with and without corn oil. The DNA was extracted and sequenced using illumina miseq platform. The DNA sequences were compared to ARG-ANNOT and Res finder databases to predict the presences of antimicrobial resistance genes. Results: The microbiome was distributed among 26 different bacterial genera, the read counts were used to estimate the relative abundance with Lactococcus (0.0-89.2) and Enterococcus (0.2-43.2) being the most predominant genera after 24h. The relative abundances of the other genera were Acinetobacter (0.0-20.2), Escherichia (1.8-17.3), Enterobacter (0.0-15.6), Klebsiella (0.0-10.8), Cronobacter (0.0-0.2) and Citrobacter (0.0-4.9). Twenty two Antimicrobial Resistance Genes (AMR) were identified and the mean number of AMR genes identified per sample were: 10.5±3.6, and the most abundant classes of antibiotic were β-lactam (blaADC-25, blalLEN2, blalOXA-51, blalSHV-12, blalFOX-1, blalLEN2, blalACC-2 and blalCMY-18) and tetracycline (tet(H), tet(S), tet(39), tet(M), tet(L), tetB(P), and tetA(P). Resistance genes to fosfomycin (fosA), macrolide (lsa(A), quinolone (oxB), sulphonamide (slu2) and trimethoprim (dfrA7) were also observed. Conclusion: Overall, relative abundance of microbiome and their associated resistome suggests the need to consider meat based foods that are served raw or undercooked as a potential reservoir for the spread of food borne pathogens and antibiotic resistance genes.
Abstract Title:
An Assessment of the Pollution and Plasmid-Mediated Multidrug Resistant Bacterial Status of Ikpoba River in Benin City, Nigeria

Primary Author Block:
A. R. Akpe, G. I. Okwu, I. J. Femi; Ambrose Alli Univ., Ekpoma, Nigeria

Abstract Body:
Background: River water is a major source of water for household use in most rural communities in Nigeria. River pollution refers to the contamination of rivers which occur when waste and different other pollutants are discharged into river without being properly treated. Studies on river water pollution and their implication to public health has been ongoing. Methods: Standard microbiological and physicochemical methods were used to determine the physicochemical parameters, microbial density and diversity of samples, antibiotics susceptibility patterns and plasmid profile of bacterial isolates. Results: Results showed that the density of the microbial isolates was highest during the dry season. There is significant difference (P>0.05) in the heterotrophic microbial counts in the dry and wet seasons which ranged from 1.23±0.15 x 102 cfu/ml to 5.80±20.00 x 104 cfu/ml. The highest counts were at the point of waste discharge while the lowest counts were recorded from the upstream samples. The most probable number of coliforms ranged from 8.00±1.00/100ml to 47.00±3.00/100ml. The diversity of the microbial species was more in the wet season than in the dry season. The microbial isolates were E. coli, Salmonella sp, Vibrio sp, Staphylococcus aureus, Streptococcus faecalis, Aspergillus fumigatus, Aspergillus niger, Penicillium sp and Rhizopus sp. The results of the physicochemical analysis showed a pH range of 5.3 to 9.5. Other physicochemical parameters such as organic matter content, turbidity, BOD5 and salinity particularly at the point of effluent discharge and downstream were higher during the dry season. The antibiotics susceptibility testing of the bacterial isolates revealed a multidrug resistant status for Staphylococcus aureus and Streptococcus faecalis. The plasmid profile of these multidrug resistant isolates was determined and results revealed that both isolates harbouring plasmid of size 4.5 kb. Antibiotic susceptibility of the isolates when cured of plasmid revealed loss of resistance to over 75% of the antibiotics they were originally resistant to. Conclusions: The microbial and physicochemical properties of the river showed that it is unfit for human consumption. The microbial density is higher in the dry season and lower in the wet season while there are more microbial diversities in the wet season than in the dry season. Also the presence of plasmid-mediated multidrug resistant bacteria in the river water samples is a threat to chemotherapy. The public health implications of these findings is hereby highlighted.
Abstract Title:
Prevalence and Molecular Characteristics of Extended-Spectrum Beta-Lactamase (Esbl)-Producing Escherichia coli in Commercial Cattle Farms

Primary Author Block:
S. Lee1, L. Teng1, H. Kim2, K. C. Jeong1; 1Univ. of Florida, Gainesville, FL, 2KyungHee Univ., Yongin-si, Korea, Republic of

Abstract Body:
Extended-spectrum β-lactamase (ESBL)-producing Escherichia coli (ESBL-E. coli) have been rapidly disseminated intercontinentally through complex transmission routes with diverse reservoirs. Clinically relevant ESBL-E. coli strains have been found in food producing animals, indicating food animals are important reservoirs of ESBL-E. coli. However, ESBL-E. coli in beef cattle raised on grass-feeding cow/calf operations are rarely studied, and limited information is available for the occurrence of this antimicrobial resistant microorganisms. We collected 1,096 samples from 17 commercial beef farms in Florida including feces of calves and cows, soil, water, and forage to understand the occurrence of ESBL-E. coli and to characterize the ESBL-E. coli isolates. Eleven farms had ESBL-E. coli in the majority of samples (animals, forage, and soil) except water samples, and the prevalence of ESBL-E. coli ranged between 1.85 to 19.6%. The average prevalence and concentration were 7.42% and 1.56 log CFU/g of feces (95% CI: 1.37-1.74), respectively. To get insights into the potential virulent properties in ESBL-E. coli, we examined the 59 ESBL-E. coli isolates using whole genome sequencing and comparative genomics. CTX-M (66%, 39/59) gene was the most predominant ESBL gene type and TEM-type ESBL gene was also encoded in 54% (32/59) of the isolates. Results from the antibiotic resistance genes (ARGs) profiling showed that all isolates were multidrug resistant and the functionality of identified ARGs was confirmed by antibiotic susceptibility test, indicating all strains were resistant against at least 4 different antibiotics. Furthermore, we found that all ESBL-E. coli isolates shared virulent factors related to adherence, invasion, ion uptake, and bacterial secretion systems, suggesting pathogenic potentials of ESBLs. Phylogeny analysis based on core genome alignment with 59 ESBL-E. coli strains made non-specific clusters regardless of the farm location, suggesting ESBLs in beef cattle might have introduced outside rather than in-housed-raised. Our results indicate that ESBL-E. coli are prevalent on cow/calf operations regardless of antibiotic use and they are globally transmitting by carriers located nearby farm area.
Abstract: The use of antimicrobial drugs within animal-based agriculture and clinical settings continues to raise public health concerns over selection for antimicrobial resistant (AR) bacteria. Concern is further heightened by growing evidence that residual antibiotics and AR bacteria may leach from anthropogenic sources into natural ecosystems, creating novel opportunities for the evolution, maintenance, and dispersal of AR genes within bacteria populations. However, we know relatively little about the geographic distribution of AR in natural environments and how specific anthropogenic sources of AR contribute to emergence in wildlife and environmental reservoirs. Recent advances in metagenomic sequencing technology allow for characterization of the AR potential of a microbial community and linkage of specific microbial taxa to particular AR genes of interest. Here, we use metagenomics to explore spatial patterns of AR genes from two owl species-Bubo virginianus and Strix varia-that may serve as sentinels of AR in the natural environment. We collected cloacal swabs from 80 owls recovered throughout Minnesota and identified enterobacteria-associated AR genes using shotgun metagenomic sequencing. We then applied tools from spatial epidemiology to investigate how anthropogenic landscapes-both agricultural and urban-shape the distribution of AR genes. Further, we compared owl AR genes to those found in 23 samples from commercial poultry farms to evaluate the extent of AR gene sharing between agricultural and wildlife samples. Preliminary data indicate that AR gene prevalence in wild owls was relatively low compared to levels found in other studies on raptors. However, among owls that did exhibit AR, we detect genes conferring resistance to a range of antibiotic classes, including clinically relevant extended-spectrum beta-lactamases. Additionally, over 60% of AR genes found in owls are also identified in poultry samples, suggesting a possible link between agricultural AR sources and wildlife. Our results establish baselines for monitoring the distribution of AR in natural environments and provide insight into the role anthropogenic sources may play in AR gene dissemination.
Abstract Title:
Isolation and Molecular Characterization of Multiple Antibiotic Resistant Bacteria from Finfish and Shellfish Aquaculture Environment

Primary Author Block:
A. Hossain; Graduate Sch. of Environment and Information Sci., Yokohama, Japan

Abstract Body:
Background: Finfish and shellfish aquaculture have led to a growing problem with the bacterial infections and the treatment requires the intensive use of antibiotics. Exposure to high antibiotics might induce multiple resistances in bacteria and antibiotic resistance genes (ARGs) in aquaculture environment. The ARGs have been recognized as a new emerging contaminant in environment. Therefore, the present study was designed to isolate and molecular characterize the multiple antibiotic resistant bacteria from finfish and shellfish aquaculture water samples and evaluate their distribution in the study areas.

Methods: Water samples (n=30, 15 finfish and 15 shellfish aquaculture water samples) were collected from six distinct sites of Bangladesh. Nutrient agar (NA) medium was used to isolate the bacterial strains from the samples. Antibiotic susceptibility test (16 different antibiotic discs; Oxoid, UK) was conducted by using the standardized discs agar (using MHA) diffusion method. The identification of multiple antibiotic resistant bacteria was carried out by 16S rRNA gene amplification using PCR and confirmed by sequencing.

Results: The results of the bacterial plate count were ranged from 1.04 to 12.21 × 10² cfu per ml of water samples. Twenty nine bacteria isolates were selected for antibiotic susceptibility test. The overall prevalence of multiple antibiotic resistant bacteria (at least 3 antibiotics resistant) in the present study was 51.7% (15 out of 29 isolates). The finfish aquaculture water samples (9 isolates) showed the higher resistant pattern than that of shellfish aquaculture (6 isolates). Among the characterized isolates, Vibrio cholerae showed the highest resistant pattern against 13 tested antibiotics followed by Exiguobacterium spp., and Vibrio fluvialis.

Conclusion: The higher multiple antibiotic resistances (51.7%) in bacteria could be associated with the indiscriminate application of antibiotics in finfish and shellfish aquaculture and might be a potential threat to culture species as well as human health.
Abstract:
Free living birds can be significant contributors of antibiotic resistant bacteria (ARB). A constructed wetland, where ~15,000 American crows (Corvus brachyrhynchos) roost between autumn and spring, was sampled on the University of Washington Bothell Campus for the presence of Antibiotic resistant E. coli (ARE). Crow droppings from individual birds and grab samples of water were collected in 2014-2015 from different sites in the wetland influenced either by North Creek, campus run-off or surface water. E. coli were isolated from 49 of 61 samples by selective agar plating. Water samples were collected from these sites during 2016, to determine storm water contribution of ARE. A total of 98 fecal and 155 water E. coli isolates’ susceptibilities were tested against 13 antibiotics using the Kirby Bauer method. Antibiotic resistance (AR) to ampicillin (59%), amoxicillin-clavulanic acid (54%), streptomycin (49%), nalidixic acid (NA) (48%), neomycin (N) (38%), ceftiofur (19%) and tetracycline (Tc) (17%) was identified in the fecal isolates and ~20% were multidrug resistant (MDR). Water isolates displayed similar AR pattern but had less percentage of ARE relative to the feces; further testing is needed for verification. Water samples collected during storm events had ~twofold increase in S, NA and (Tc) resistant E. coli. Tc resistant isolates frequently carried tet(A) (33%) or tet(B) (23%) genes. Presence of Extended Spectrum b-lactamase (ESBL) containing E. coli was determined using selective plating and verification of specific genes by PCR. The blaCTX-M gene was found in 16 water and 7 fecal isolates. blaCMY-2 gene was also present in 4 of the fecal isolates carrying blaCTX-M. All ESBL containing isolates were MDR, with 10 being resistant to NA, Tc and to S or N. Multilocus Sequence Typing analysis (MLST) identified three crow ESBL E. coli belonging to the internationally distributed ST131 clone. ST131 is highly virulent, and usually multidrug resistant; it is responsible for ~20% of the ESBL isolates globally. Two non-ESBL isolates with the exact same AR pattern, one each from water and feces, belonged to the human clone ST58. Phylogenetic analysis of the crow isolates by a PCR method, showed 37% to belong to the commensal strain phylo-group B1, and included the ST58 isolates. The B2 group, to which most extra-intestinal pathogenic E. coli belong, was the next most common (21%) and included the ST131 strains. This study demonstrates that American crows can acquire human associated ARB, including virulent ESBL strains, and act as reservoirs for these strains.
Abstract Title:
Impact of Wastewater Treatment on the Prevalence of Integrons and Genetic Diversity of Integron Gene Cassettes

Primary Author Block:
X-L. An1, Q-L. Chen1, D. Zhu1, Y-G. Zhu1, M. Gillings2, J-Q. Su1; 1Inst. of Urban Environment, Xiamen, China, 2Dept. of Biological Sci., Sydney, NSW, Australia

Abstract Body:
The integron platform allows the acquisition, expression, and dissemination of antibiotic resistance genes within gene cassettes via horizontal gene transfer. Wastewater treatment plants (WWTPs) contain abundant resistance genes; however, the knowledge about the impacts of wastewater treatment process on integrons and their antibiotic resistance gene cassettes is limited. In the present study, we used the combination of clone library and high throughput amplicon sequencing analysis to investigate the abundances of class 1, 2 and 3 integrons and their corresponding gene cassette contents in WWTPs. Our results showed that class 1 integrons were most abundant in WWTPs and wastewater treatment process significantly reduced the abundance of all integrons. Influent harbored the highest diversity of class 1 integron gene cassettes, whereas class 3 integron gene cassettes exhibited highest diversity in activated sludge. Sixteen unique gene cassette arrays were observed in class 1 integrons, most of which were novel. Aminoglycoside, beta-lactam and trimethoprim resistance genes were prevalent in class 1 integrons, while class 3 integrons mainly carried beta-lactam resistance genes. A core class 1 integron resistance gene cassette pool persisted during wastewater treatment, implying that these integron-mediated resistance genes could have high potential to spread into environments through WWTPs. These data provide new insights into the impact of wastewater treatment on integron pools and highlight the need for surveillance of resistance genes within both class 1 and 3 integrons.
Abstract Title:
Antimicrobial Susceptibility Profiles of Coliform Bacteria Isolated from Fresh Vegetables, Agricultural Soil, Livestock and Selected Rivers in the Eastern Cape, South Africa

Primary Author Block:
A. I. Okoh, L. Mpondo, A. Ngomti, Z. Ntshanka, C. D. Iwu, M. A. Adefisoye; Univ. of Fort Hare, Alice, South Africa

Abstract Body:
Background: The evolution and spread of drug resistance is a crucial global challenge limiting the number of effective antibiotics for treating infections with grave implications for human health and economy. This study evaluates the antibiogram profiles of E. coli recovered from fresh vegetables, agricultural soil, livestock and their waste products and selected rivers in the Eastern Cape, South Africa

Method: Fifty samples (8 river water, 2 soil, 10 rectal swab, 10 nasal swab, 10 faecal and 10 fresh vegetables) were collected in October 2017 and analysed using standard methods. Polymerase Chain Reactions (PCR) were used to identifying E. coli isolates (uidA gene, 147 bp) while disc diffusion method was used to determine the antibiotic susceptibility pattern of the isolates against a panel of 10 antibiotics classes according to the Clinical Laboratory Standards Institute guildelines

Result: Results showed E. coli counts for water samples ranged from 1.5 × 10¹ to 2.0 × 10² CFU/100 ml while the faecal samples had counts ranging from 3.6 × 10² to 7.6 × 10¹ CFU/100 ml. E. coli was not detected in the vegetable samples while 2 CFU/g was recorded for one of the soil samples. PCR analysis of randomly selected presumptive E.coli isolates gave positive rate of 94% (262/278). All test isolates showed 100% (200/200) resistance against teicoplanin while the isolates exhibited resistance rate of 99.5%, 97.55 and 74% against linezolid, vancomycin and erythromycin respectively. Resistance rates ranging from 1% to 28% were recorded for other classes of antibiotics. Multiple antibiotic resistance (resistance to 3 or more antibiotics) was exhibited by 29.2% of the test isolates with the commonest multiple antibiotic resistance phenotype (MARP) being E-LZD-TEC-VAN (21 isolates) while the multiple resistance indices (MARI) for the isolates ranged from 0.3 to 0.7

Conclusion: The results suggest that the agroecosystem may be a reservoir for the emergence and dissemination of antimicrobial resistance which may be transferred to human population along the food chain thus presenting a serious health challenge

Keywords: Antimicrobial, resistance, agroecosystem, public health, food chain
Session Title: AES03 - Antimicrobial Resistance in the Environment: Revealing the Resistome
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Abstract Control Number: 3750
Poster Board Number: FRIDAY - 787

Abstract Title:
Prevalence of Antibiotic Resistance in Bacteria Exposed to Perfluorinated Compounds versus Bacteria from Pristine Environments
Primary Author Block:
C. A. Mirto, A. M. Spain; Ferris State Univ., Big Rapids, MI
Abstract Body:
Perfluorinated compounds (PFCs) are persistent and recalcitrant chemicals which bioaccumulate in higher organisms in the food chain; however, their effects on microbial populations have not been well documented. These experiments investigated whether the presence of PFCs in aquatic environments select for antibiotic resistance genes in bacteria isolated from three locations in Oscoda, MI. Aquatic and sediment samples were collected from Clark’s Marsh, a highly contaminated site, with PFC concentrations exceeding 5,000 parts per trillion (ppt). Samples were also collected from a moderately contaminated site along the Au Sable River (0-40 ppt), and from a pristine site at Foote Dam Pond Overlook (0 ppt). Sixty bacterial isolates (20 from each location) were collected and tested in triplicate for resistance to seven antibiotics: aztreonam, ciprofloxacin, sulfamethoxazole/trimethoprim, ceftriaxone, tetracycline, ampicillin, and polymyxin B. No difference in the rates of antibiotic resistance between PFC-contaminated and pristine environments was observed. However, 81.6% of all isolates tested displayed resistance to at least one antibiotic, indicating aquatic environments may serve as reservoirs for antibiotic resistance genes, regardless of PFC contamination.
Session Number: 76
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Abstract Control Number: 7052
Poster Board Number: FRIDAY - 788

Abstract Title:
Functional Screening and Frequency of Triclosan Resistance in Wastewater and Clin. Microbes

Primary Author Block:
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Abstract Body:
Background: The synthetic biocide triclosan (TCS) is widespread in modern consumer products, and is a frequent contaminant in wastewater treatment plants (WWTPs). The antimicrobial properties of TCS have led to concern that exposure may impact resistance to clinically important antibiotics. This study aimed to functionally identify mechanisms of TCS resistance (TCSR) in a WWTP metagenome and to assess TCSR frequency in WWTP-derived and clinical isolates of Enterococcus sp. and E. coli. Methods: Metagenomic DNA was extracted from influent biomass harvested from a Calgary, AB WWTP from 2015-2016. Large-insert (>38kb) DNA was introduced into pCC2FOS for construction of a pooled cosmid library (>105 clones). For TCSR screening, OD-standardized library and empty vector E. coli cultures were enumerated on Mueller-Hinton agar with or without TCS (5 μg/mL). Unique TCSR cosmids were sequenced by Illumina Miseq. In tandem, isolates of Enterococcus sp. (n=135) and E. coli (n=101) were collected from the same WWTP influx. Comparator clinical E. coli (n=25) and Enterococcus sp. originating from sterile (n=42) or non-sterile sites (n=169), or designated as vancomycin-resistant Enterococci (VRE, n=149) were collected from Calgary hospitals. Susceptibility profiles (12-drug panels) were generated via disk-diffusion. The TCS MIC was determined via microtitre broth dilution assay. Results: TCSR clones (MIC: >64 μg/mL) comprised 0.06% of the cosmid library. TCSR mechanisms on clones with identity to Aeromonas sp. included a putative multidrug transporter with 59% aa identity to the Pseudomonas aeruginosa MexF efflux pump. Another cosmid encoded genes involved in lipopolysaccharide modification, likely originating from E. coli. The TCS MIC90 for each species tested was determined: the MIC90 for E. faecalis, E. faecium, and E. coli was 8.0, 8.0, and 0.06 μg/mL, respectively. Modal distributions for Enterococcus sp. revealed no evidence for TCSR. In contrast, some E. coli isolates (2/25 clinical isolates; 4/101 WWTP isolates) had MICs outside the modal distribution (MICs: >1 μg/mL ). TCSR E. coli isolates were resistant to ampicillin and to ≥ 3 antibiotics, and 5/6 isolates were putative extended spectrum β-lactamase-producers. Conclusion: Functional screening revealed two putative mechanisms of TCSR in wastewater microbes. The absence of multimodal MIC distributions in Enterococcus sp. indicates that TCSR is uncommon in those populations. Despite this, TCSR occurred at low frequency in the clinical and WWTP E. coli isolates tested.
Extended-Spectrum β-Lactamase-Producing Escherichia coli from Meconium of Newborn Calves

Primary Author Block:
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Abstract Body:
Extended-spectrum β-lactamase (ESBL)-producing Escherichia coli has become a great concern to public health primarily because of its resistance to third-generation cephalosporins, which are widely used in human healthcare facilities to treat bacterial infections. Although it is controversial, it is commonly believed that food animals acquire antimicrobial resistant (AMR) bacteria by receiving antibiotic treatments. The purpose of this study was to identify the earliest time when animals are exposed to ESBL-producing E. coli. Meconium samples were collected from the rectal anal junction of 322 newborn calves. ESBL-producing E. coli were identified from the samples by plating on MacConkey agar supplemented with Cefotaxime (4 µg/mL). Isolates were further characterized with ChromAgar E. coli and CTX-M gene typing using PCR. ESBL-producing E. coli was detected in 7.5% (24/322) of meconium samples of newborn calves. Illumina MiSeq platform was employed for Whole Genome Sequencing (WGS) of 37 strains from 24 calves. After assembly, nineteen representative strains were selected, based on their Sequencing Types (STs) and whole genome architecture, for further bioinformatics analyses and antimicrobial susceptibility test. Following WGS, phylogenetic analysis revealed that these strains clustered into 8 clusters that coincided with globally prevalent STs. All the isolates carried a variety of virulence genes and were resistant to multiple antibiotics. Comparative genomics analysis revealed that ESBL-producing E. coli from meconium harbored unique efflux pump genes and higher copy number of antibiotic resistant genes compared to isolates from cows. In particular, we identified hyper-virulent strains of ST117 that carries Shiga toxin-encoding genes (stxAB), which may cause severe human diseases. This is the first study showing the prevalence ESBL-produced E. coli in meconium of newborn calves, indicating animals are even start to be exposed to AMR bacteria in the uterus.
Abstract Title:
Effect of Heavy Metals on Antibiotic Resistance in Wastewater Treatment Plants
Primary Author Block:
F. Barancheshme, A. Alansari, J. Amburgey, M. Munir; Univ. of North Carolina at Charlotte, Charlotte, NC
Abstract Body:
The occurrence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in our environment is a growing global health problem. Bacterial infections and serious outbreaks are even more deadly if they are associated with antibiotic resistance. This study aims to understand the development of ARGs in wastewater treatment plants (WWTPs). Presence of metals in wastewater treatment plants could be one of the factors responsible for selection of antibiotic resistance among exposed bacteria in the environment. A relationship between heavy metal residues and selection of ARB and ARGs is hypothesized. The main goal of this manuscript is to determine the effect of heavy metal as a stress and on selection of the ARGs. This study investigates the relationship between exposure level to metals and selection of antibiotic resistance in wastewater treatment plants. To achieve this goal, activated sludge bioreactor was built and wastewater from activated sludge tank of a WWTP was sampled and exposed to different levels of copper and zinc. The copies of tetracycline resistance gene (tet A) and sulfonamide resistance (sul1) were quantified by molecular method using Quantitative Polymerase Chain Reaction (qPCR). This study will demonstrate that the occurrence of antibiotic resistant genes in the wastewater could be correlated with the presence of heavy metal in the wastewater. The results of this study may contribute to better understanding of the selection of the ARGs in wastewater treatment plants.
Abstract Title:
Multidrug Resistant Vibrio Species and Plesiomonas Shigelloides Recovered from Selected Rivers in Southwestern Nigeria: A Publ. Hlth. Concern

Primary Author Block:
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Abstract Body:
Pathogens that cause infectious water-related diseases such as diarrhea and cholera are beginning to manifest with unusual antibiotic resistance characteristics and virulence, posing a huge threat to public health. In sub-Saharan Africa region, especially Nigeria, lack of clean water has increased the exposure of communities to multi-drug resistant water-borne pathogens. This study therefore investigated the prevalence of multi-drug resistant Vibrio species and Plesiomonas shigelloides in selected rivers in Southwest Nigeria. Water samples from four different rivers were analyzed by membrane filtration method. Presumptive Vibrio species (n=315) and P. shigelloides (n=66) were further characterized by simplex PCR. Isolates were profiled for antibiotic susceptibility and resistant strains were further screened for presence of selected antibiotic resistant determinants. The data obtained were analyzed for possible associations between the resistance determinants using Pearson's chi-square exact test. The prevalence and distribution of selected resistance determinant were obtained as follows for both organisms. For P. shigelloides: sulfonamides (sulI (18%), sulII (20%), beta-lactams; (ampC 37%), tetracyclines (tetA (78%), tetE (57%). Likewise for Vibrio species: sulfonamides (sulI (19%), sulII (33%), beta-lactams; (ampC 39%; blaOXA (27%), blapse (11%), tetracyclines (tetA (28%), tetE (20%), tet39 (8%) respectively. Our findings indicated unexpected high prevalence of multi-drug resistant Vibrio species and P. shigelloides towards commonly prescribed antibiotics which suggests the need to include resistance genes surveillance in surface water monitoring and assessment programs to safeguard the public health.
Abstract Title:

Spread of Colistin-Resistant Gram-Negative Rods Through Raw and Treated Sewage from Clinical, Community and Animal Sources At São Paulo, Brazil

Primary Author Block:


Abstract Body:

Background: Polymyxins are currently being used as last-choice treatment options for multidrug-resistant infections. Unfortunately, resistance to these compounds emerged through the production of phosphoethanolamine-transferases encoded by mcr genes located in plasmids. Sewage from varied sources can be a reservoir for antimicrobial resistance, so the aim of this study was to isolate colistin-resistant Gram-negative strains from clinical, community and animal sewage sites, and assess their susceptibility profile and the presence of mcr and other resistance-encoding genes. Methods: Fifty 250-mL sewage samples were collected from human and veterinary hospitals, an animal husbandry and from Sewage Treatment Plants (STP), including raw sewage, treated effluents and reuse water. Samples were filtered through 0.45µm membranes, cultivated overnight, and resistant strains were selected on MacConkey broth containing Colistin (4µg/mL). Antimicrobial susceptibility was assessed according to CLSI 2017, and the search for genes mcr-1, mcr-2, mcr-3 and genes encoding resistance for other antimicrobial classes, was carried out by PCR and sequencing. Results: Sixty-nine colistin-resistant strains were recovered, from which 19 (27.5%) carried mcr-1 and 3 (4.3%) carried mcr-3. MCR-1-producing strains were found in all sampling sites, except for reuse water, and MCR-3 was detected in treated effluents and animal husbandry. Co-carriage of other resistance determinants was also detected: quinolone-resistant strains (animal husbandry, human and veterinary hospitals) carried qnrS (n=4) and qnrB (n=1), while beta-lactamase-producing strains carried blaCMY (n=1, animal husbandry) and blaCTX-M (n=1, veterinary hospital). One strain from the veterinary hospital co-carried mcr-1, qnrS and blaCTX-M. Conclusions: Results show sewage as a reservoir of strains carrying resistance to the last therapeutic options for severe infections. Collection and treatment of sewage in Brazil is deficient, and the resistant population of bacteria that survive treatment systems will be discharged in water bodies and restart the spread cycle. Efforts need to be driven to reduce resistance rates in all fields, with strong policies of rational and restricted use of antimicrobials.
Abstract Title:
Urban Streams As Reservoirs for Dissemination of Carbapenem Resistant Bacteria

Primary Author Block:
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Abstract Body:
Background: The prevalence of multi-drug resistance bacteria in surface waters represents a serious threat to public health. The spread of carbapenem resistant bacteria/genes in the urban aquatic environment have been mainly attributed to the discharge of wastewater effluent or sewage into receiving waters. Therefore, urban streams impacted by fecal pollution are at higher risk of contamination. This study examines the occurrence of carbapenem resistance bacteria and genes in urban streams. Methods: Surface water samples were collected from 13 sampling points in Proctor Creek watershed, a highly urbanized watershed in Atlanta, GA with a long history of fecal pollution from human sewage. Carbapenem resistant bacteria were isolated from surface waters using ESBL/meropenem plates. Bacterial isolates were identified by sequencing of 16S rRNA genes. Antibiotic susceptibility tests were performed to determine resistance of isolates to beta-lactam antibiotics including the carbapenems. Putative resistant genes were detected using previously published PCR primers. Results: Results showed the presence of carbapenem resistant bacteria on the main stem and tributaries of Proctor Creek. Bacterial species identified through sequencing of 16S rRNA genes include strains of Stenotrophomonas maltophilia, an opportunistic pathogen that showed 100% resistance to the carbapenems meropenem and ertapenem. About 65% (28/43) of isolates were identified as S. maltophilia. The beta-lactamase encoding genes blaL1 and blaL2 were observed in S. maltophilia isolates, showing broad spectrum of activity against carbapenem antibiotics, while 60% showed susceptibility to ceftazidime. Other resistant bacteria of significance include several species of Pseudomonas, Chryseobacterium and Elizabethkingia. Within these groups of bacteria are opportunistic pathogens such as E. anopheles and E. meningoseptica that have been responsible for disease outbreaks in the U.S and other parts of the world. Conclusion: This study shows the widespread occurrence of carbapenem resistant bacteria in urban waters and supports efforts to track the sources of resistant bacteria in the environment.
Abstract Title:
Captive Baboons Harbor Enterococcus Resistance Genes

Primary Author Block:
E. S. Mitema, J. P. Mwova, G. Aboge; Publ. Hlth., Pharmacology and Toxicology, Nairobi, Kenya

Abstract Body:
Background: Captive baboons are regularly used by researchers in Kenya and other countries yet the potential risk of transfer of enterococcal antibiotic resistance genes from baboons to humans is unknown. This study investigated whether enterococcal isolates from baboons are phenotypically resistant to selected antibiotics and also harbor antibiotic resistance genes. Methodology: Identification of Enterococcus spp was done on selective media (Slanetz and Bartkey) and confirmed by PCR using specific primers. A total of 73 enterococci isolates from feces of captive baboons (Papio Anubis) were subjected to antimicrobial susceptibility testings. Six commonly used antibiotics - ampicillin, vancomycin, doxycycline, erythromycin, levofloxacin and linezolid were assayed by disk diffusion method employing Clinical and Laboratory Standards Institute (CLSI) guidelines. A conventional polymerase chain (PCR) reaction targeting the 16S rRNA gene was used to confirm Enterococcus spp and screen for ermB, ermA, tetL, tetM and tetO resistance genes. The resistant amplicons were sequenced, analyzed and thereafter BLAST analysis performed. Sequenced resistance genes were submitted to NCBI GenBank for assignment of accession numbers

Results: Overall, 28 (38.3%) enterococcal isolates were phenotypically resistant to erythromycin and doxycycline with 26 (92.8%) and 2 (7.1%) isolates revealing resistance to erythromycin and doxycycline respectively. Enterococci were not phenotypically resistant to the other four antibiotics. Of the 26 erythromycin resistant isolates, ermB was detected in 5 (19.2%) isolates. For doxycycline, all the two isolates harbored tetL while one isolate had tetM. One isolate however, harbored ermB, tetL and tetM genes. The ermB sequences were 100 % identical to the E. fecium ermB available in the NCBI GenBank while tetL and tetM sequences were 100 % identical to either E. fecium or E. faecalis- tetL and tetM. Sequence analysis revealed that ermB was present in plasmids while tetL and tetM were located in plasmids and transposons. The ermB, tetL and tetM found in enterococci from baboons were similar to human isolates suggesting a possible transfer of the resistance determinants

Conclusions: Enterococci isolated from feces of baboons in Kenya harbor mobile antibiotic resistance genes that may be transferred to humans posing possible therapeutic challenges. Vancomycin and linezolid may still be useful antimicrobial agents in the treatment of enterococcal infections in Kenya since no resistances were detected
Abstract Title:
Spread of Emergent Resistance Genes Through Sewage from Varied Sources in Brazil: A Metagenomic Approach for Hospital, Animal Husbandry and Domestic Raw and Treated Wastewater Samples

Primary Author Block:

Abstract Body:
Background: Multidrug resistance in Gram-negative bacteria has led to the re-introduction of long-used antimicrobials like polymyxins and fosfomycin as last-choice treatment options for severe infections. However, transferable resistance to these compounds has emerged and the spread of phosphoethanolamine transferase (mcr) and fosfomycin-modified (fos) genes have been increasingly reported. As most bacteria are not cultivable, this study aimed to assess the occurrence of mcr, fos and other resistance genes in sewage samples from several sources, using a culture-independent approach.

Methods: Fifty-six 500-mL sewage samples were collected from: three Sewage Treatment Plants (STP), including raw sewage, treated effluents and reuse water; one human hospital; one veterinary hospital; and one animal husbandry. Samples were filtered through 45µm membranes and DNA was directly extracted using a commercial kit. PCR and sequencing were carried out for the search of mcr-1, mcr-2, mcr-3, fosA, fosA3 and fosC2 genes, as well as for emerging RNA metilases, extended-spectrum beta-lactamases and carbapenemases. Results: MCR-1 was detected in 19 (34%) samples, from animal husbandry, STPs (reuse water included) and human hospital. MCR-2 was detected at animal husbandry, in two (3,6%) samples, while MCR-3 (55%) and FOS-A (16%) were detected at all sampling sites. RNA metilases RmtD and RmtG were both detected in 14% of samples, at animal husbandry and human hospital. Regarding beta-lactamases, CTX-M was present in 80% of samples, at all sampling sites, while carbapenemases (KPC, OXA-48, NDM, IMP, VIM) were detected in 45% of samples, except in reuse water and veterinary hospital sewage. Conclusions: Results show the spread of endemic and emergent antimicrobial resistance genes through sewage from varied sceneries, including treated wastewater that will be reused or discharged in natural water sources. This is also the first report of MCR-2 in Latin America, and the first description of RmtG metilase genes at animal husbandry. Prevention of antimicrobial resistance spread should focus on the awareness that all fields are responsible and need to establish effective policies to reduce this growing public health problem.
Session Title: AES03 - Antimicrobial Resistance in the Environment: Revealing the Resistome

Abstract Title: Are There Differentially Abundant Antibiotic Resistance Genes (Arg) Associated with Chlorinated Drinking Water Sys. As Compared to Non-Disinfected Systems?

Abstract Body:
Antimicrobial resistance (AMR) challenges clinical treatment and the economy, it has therefore been deemed the greatest and most urgent global risk of our time. This issue is aggravated by misuse of antibiotics and anthropogenic release of bioactive compounds into the environment. Engineered systems for water treatment such as wastewater, and drinking water are thus critical points of AMR dissemination into the urban water microbiome. AMR in the wastewater context has been widely characterized, however their dynamics in drinking water systems has been poorly studied. Use of chlorination for water disinfection has been hailed as one of the 20th century’s greatest public health achievements. Disinfectant residuals are used to manage microbial growth in distribution systems, however they may introduce additional concerns in terms of health (e.g. disinfection by products (DBPs)). Concerns about DBP health risks have prompted a shift towards non-disinfected systems in Europe. In order to compare and contrast the current situation of AMR in the drinking water microbiome, samples were collected chlorinated (Dis) and non-chlorinated (nonDis) systems from England, Scotland, and Netherlands. Water quality data was gathered (i.e. pH, DO, total chlorine, temperature), and samples were filtered on 0.2-micron filters to harvest bacterial cells for DNA extraction. Metagenomic sequencing of the extracted DNA was performed to obtain paired-end reads for the non-targeted detection of ARGs. Resulting reads were co-assembled into scaffolds using metaSPAdes, followed by gene calling using prodigal, which allowed for annotation against the Comprehensive Antimicrobial Resistance Database with a stringent reciprocal BLAST approach. Metagenomic sequencing indicates a high diversity of AMR traits, with over 400 antibiotic resistance ontologies (ARO) across assemblies. These AROs are broadly classified as efflux pumps. The most represented antibiotic class within all assemblies is glycopeptides. Preliminary results indicate that AROs related to efflux resistance mechanisms were mostly present in Dis systems, AROs associated with target protection mechanisms were unique to Dis systems, whereas AROs associated with alteration of cell wall charge and molecular bypass mechanisms were unique to nonDis systems. On-going efforts using genome binning, will attempt to contextualize these genes in terms of their host to determine their presence/absence in opportunistic pathogens and assess ARG associated risks from a public health perspective.
Antibiotic Resistance Profiles in Sewage Across the United States

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Abstract Body:
The ubiquitous use of antibiotics in medicine and agriculture has led to widespread development of microbial antibiotic resistance. Sewerage systems act as a collector of human and agricultural waste; therefore, antibiotic resistant bacteria are thought to be abundant in wastewater. Aging infrastructure in the U.S. has created a significant conduit for dissemination of untreated wastewater to natural waterways, and large rain events regularly overflow combined stormwater-sewage systems. Consequently, antibiotic resistant bacteria and antibiotic resistance genes (ARGs) are discharged regularly into the environment. However, little is known about the diversity, abundance, and geospatial differences of ARGs in untreated sewage. To establish a baseline for geospatial resistance variation in untreated sewage, we used droplet digital PCR (ddPCR) to quantify three ARGs (sul1, tetO, and qnrS) and a class 1 integron-integrase gene (intI1) within wastewater influent from 24 cities across the United States. Gene abundances ranged from 104 to 107 copies per mL, which scales to 1017 copies of a single ARG entering a single wastewater treatment plant each day. Gene quantification also indicated differences in gene profiles among cities. We find ddPCR is an effective method for quantifying target genes in sewage due to its simplified quantification, increased signal-to-noise ratio, and consistency in measurements.
Session Title: AES03 - Antimicrobial Resistance in the Environment: Revealing the Resistome

Abstract Title: High Incidence of Sulfamethoxazole-Trimethoprim (Sxt) Resistance in Stenotrophomonas Maltophilia Isolated from the Environment in Mexico

Abstract Body:
Background: Stenotrophomonas maltophilia is an opportunistic pathogen involved in the cause of diseases in immuno-compromise patients. S. maltophilia are known for their ability to quickly develop resistance to antimicrobial agents after brief contact. Several studies have associated the development of resistance in S. maltophilia to antimicrobial agents with hospital/clinical exposure, but some multi-resistant strains have been isolated outside the hospital environment. Sulfamethoxazole-Trimethoprim (SXT) is the first drug of choice in the treatment of S. maltophilia-associated infection. However, recent studies have revealed the upsurge in the rate at which hospital-originated strains of S. maltophilia resist SXT, thus attributing resistant to clinical exposure. In this light, we decided to isolate S. maltophilia from different environmental sources (Soil and Sewage) and evaluated their susceptibility pattern to different antimicrobial agents. This is to understand the influence of the Stenotrophomonas' environment on their response to antimicrobial agents. Methods: Forty-Three Stenotrophomonas maltophilia strains from the environment and 10 from the hospital were isolated from Mexico. The conventional microbiological techniques were used for the phenotypic identification of the isolates. Molecular identification of isolates was determined by amplifying the 16S rRNA gene fragment of the Stenotrophomonas' genome. The patterns of isolates’ susceptibility to antibiotics were determined using both agar diffusion and MIC (Minimum Inhibitory Concentration) evaluation technique. Results: The isolated Stenotrophomonas maltophilia strains were resistant to most antibiotics tested except ofloxacin (2.32%), ciprofloxacin (6.98%), and pefloxacin (9.3%) (fluoroquinolone) to which they show a low level of resistance. Most isolates were resistant to sulfamethoxazole-trimethoprim (SXT) (81.4%). The susceptibility pattern in S. maltophilia strains isolated from the hospital, in this study followed a similar pattern with what was observed in the isolates from the environment. This suggests that resistance to antibiotics in S. maltophilia can be induced by environmental factors. Conclusion: This result provided a good reference for understanding the pattern of resistance in S. maltophilia isolates from sewage and soil to antimicrobial agents and will also guide in the selection of drugs for treating infections from them.
Abstract Title:
Parallels among Culturable Antibiotic Resistant Fecal Coliforms and Resistance Genes from Soils Amended with Dairy Manure Or Compost During Vegetable Cultivation

Primary Author Block:
L. Wind, L-A. Krometis, W. Hession, A. Pruden; Virginia Tech, Blacksburg, VA

Abstract Body:
Identification of agricultural practices that mitigate the environmental dissemination of antibiotic resistance is a key need in preserving drug efficacy and protecting public health. We evaluated the effects of soil amendment type (inorganic fertilizer, raw dairy manure, composted dairy manure, or no amendment), vegetable type (lettuce, radish), and antibiotic use (pirlimycin and cephapirin) of cattle manure-derived amendments on the incidence of culturable antibiotic-resistant fecal coliforms through a field-scale controlled plot experiment. To reduce statistical bias associated with values below the limit of detection, zero-inflated Poisson (ZIP) regression models were used to identify significant trends in coliform count data. Antibiotic-resistant culturable fecal coliforms were recoverable from soils across all treatments immediately following application, though persistence throughout the experiment varied by antibiotic class and time. Compost-amended soils had the highest levels of cephalosporin-resistant fecal coliforms (5.64 log10 CFU/ g of soil), regardless of the antibiotic history of the cows providing the manure. Significantly, higher levels of total, ceftazidime, and erythromycin-resistant fecal coliforms were recovered from compost-amended as compared to the raw manure-amended soils (p < 0.01). Parallel quantification of resistance genes (sul1, tet(W), erm(B), intI1) was used to confirm observed culturable trends. Soils amended with raw dairy manure yielded high relative sul1 and tet(W) gene copies on Day 0, correlating with an observed spike in associated ARBs, and remained detectable for 113 and 39 days longer than resistant bacteria, respectively. Interestingly, erm(B) was not detected, despite detection of erythromycin-resistant bacteria throughout the experiment. This work is of particular interest given the relevance of fecal coliforms in tracking human pathogen risk in multiple environments (e.g., water, crops) throughout agricultural production.
Prevalence of Multi Drug Resistant Bacteria on Environmental and Medical-Device Surfaces

Abstract Body:
Background: The microbial monitoring of environmental and medical-devices’ surface is used to evaluate efficacy of routine cleaning and disinfection practices and to detect the presence of specific Nosocomial Pathogens. The prevalence of Multidrug Resistance organisms in hospital premises projects serious problems in transmitting to susceptible host which is difficult to treat. Methods: A cross sectional descriptive research was conducted from December 2016 to June 2017 at the pathology laboratory of Korea Nepal Friendship Hospital (KNFH). A total 140 samples were considered, that encompasses the medical devices of hospital (n=100), housekeeping surfaces (n=15) and air (n=25). Susceptibility test for bacterial isolates was done by disk diffusion assay. Results: Out of the total 140 samples taken and analyzed, 100% showed growth positivity. In most of the swabs taken, Coagulase Negative Staphylococci was dominant, followed by Staphylococcus aureus, Streptococcus spp., Micrococcus spp., E coli, Pseudomonas spp., Bacillus spp., Acinetobacter spp., Klebsiella spp., Fungi, and least were Proteus spp. The dry surfaces were dominantly contaminated by gram positive bacteria whereas moistened surfaces like wash basin were contaminated by gram negative as well as gram positive bacteria. Total 277 strains were exposed to various class of antibiotics, among the gram positive environmental isolates, Coagulase Negative Staphylococci 16 (34.78%) had highest MDR prevalence followed by Staphylococcus aureus 8 (29.62%), Streptococcus spp. 4 (12.90%), Micrococcus spp. 4 (9.30%) and no MDR was shown by any Bacillus spp isolates. Whereas, in case of gram negative, Klebsiella spp. 6 (35.29%) had highest MDR prevalence followed by Acinetobacter spp. 6 (31.57%), E. coli 8 (27.58%), Pseudomonas spp. 4 (18.18%), and lastly Proteus spp. with no MDR at all. Conclusions: The thick dirt covering the cotton swabs and heavy microbial load on them has displayed not only disinfecting practice is lacking but also cleaning practice is missing. Heavy contamination shows possible Nosocomial Infections breakout, it’s important to have routine microbial assessment with standard protocol and find ways to decrease its load.
Abstract Title:
Biofilms Assessment of E. coli and Salmonella Isolates from Poultry in Ilorin, Kwara State

Primary Author Block:
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Abstract Body:
Biofilms are complex exopolysaccharides which have been implicated in a variety of diseases and have
great global public health concerns. They confer advantages such as antibiotic resistance, immune
evasion and general persistence in changing and often hostile environments to microorganisms
especially in the food chain. Studies on biofilm formation by isolates of E. coli and Salmonella at Ilorin
are limited. The objective of this study was to assess the biofilm forming potentials of E. coli and
Salmonella isolates from poultry and to assess the impact of varying sugar concentrations on biofilm
mass formation. Biofilms of eight laboratory stock cultures, two each of E. coli (non-pathogenic), E. coli
O157:H7, Salmonella Nagoya and Salmonella enteritidis from poultry were retrieved and subcultured
prior to the experiment, on XLD agar and subsequently subcultured onto tryptic soy broth (TSB). Biofilm
mass was developed in TSB in 96 well polystyrene microtiter plates. The broth was supplemented
with fructose or sucrose (at 2% and 4%). Biofilm development was permitted at 37°C for 12h, 24 h and 36h
using a multifactorial study design. Un-inoculated broths and broths not supplemented with the sugar
served as controls. Biofilm masses were quantified using the crystal violet binding assay. The experiment
was done in three replicates. Statistical analysis was done using two-tailed independent Student’s T-
test whereas One-way ANOVA was used to determine the significant differences at p<0.05. The mean biofilm
mass (OD=optical density) for the E. coli isolates with 2% fructose was less (0.303±0.75) than 0.508±0.16
for 4% fructose. Similar results were obtained for 2% and 4% sucrose. However, the biofilm mass for the
Salmonella isolates with 2% fructose was higher (0.716±0.18) than the 4% fructose (0.578±0.13).
Conversely, there was a higher mass (0.53±0.14) with 4% sucrose when compared with 2% sucrose
(0.222±0.17). E. coli isolates showed the least biofilm mass with 2% fructose, while 4% sucrose showed
the highest biofilm mass. On the other hand, Salmonella isolates developed the highest biofilm mass
with 2% fructose, while the least biofilm mass was obtained with 2% sucrose. However, in both the E.
coli and Salmonella isolates, biofilm development increased steadily with incubation time. The study
indicates the presence of biofilm forming E. coli and Salmonella spp. in poultry which may be a primary
cause of antimicrobial resistance. Presence of fructose or sucrose had impact on biofilm formation by E.
coli and Salmonella spp. from poultry in Ilorin.
Biofilm Forming Potential of Free and Adherent Marine Bacteria Isolated from Coastal Waters of Karachi, Pakistan

Primary Author Block:
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Abstract Body:
Background: Biofilm formed by bacteria plays a vital role in marine environment to transfer metal/minerals from sedimentary environment to bacteria. This transformation plays an important role in marine microorganism’s growth under stressed conditions. In order to determine adherence abilities of marine bacteria living in different environmental conditions, differentiate them from others, we carried out quantifications of cell adhesion and biofilm formation by each isolate. Materials: Biofilm formation by marine isolates was studied by test tube biofilm assay method and to quantify the biofilm formation by marine isolates, microtiter plate biofilm assay was performed. A total of 197 different marine bacteria were isolated and identified from marine coastal waters of Karachi, Pakistan. They included 70 (35.53 %) free bacterial strains isolated from sea water and 127 (64.47 %) attached bacterial strains isolated from mud and rock surfaces. Results: A total of 118 (60%) out of 197 marine bacteria showed clear visibility of adherence to the surfaces of test tube. Isolated attached bacteria were more fairly adherent than free bacteria with total percentage assemblage of 67% and 33% respectively. In order to quantify the biofilm formation, marine bacteria were screened in 96 well microtitre plate biofilm assay at different incubation period and biofilm was quantified by crystal violet staining method. According to the optical density measured at 570 nm wavelength in microtitre plate after 48 hrs incubation, screened marine bacteria were classified into three groups based on absorbance at 570 nm wavelength after 48 hrs incubation, group I: (OD570 ≥ 1), 78 strains out of 197 (40%) were significantly stronger biofilm forming, group II: (0.1 ≤ OD570 < 1) 40 (20%) strains were low grade/weaker biofilm formers, and group III: (OD570 < 0.1), 79 (40%) were negative for biofilm formation. The level of biofilm formation was significantly increased (approximately doubled) from 3 to 48 hours. Three hours incubation periods was the initiation period when marine bacteria start to grow after log phase and 48 hours incubation period was the time period where the bacteria almost far more than doubled of initial bacterial cells. Conclusion: These results clearly indicate the function of biofilm formation in marine bacteria. Higher sedimentary attached bacteria showed that such surfaces plays major role in transformation of nutrients to the bacteria for their growth even under such stressed conditions.
Abstract Title:
Unique Physiologic Adaptations of Iron Reducing Extremophiles
Primary Author Block:
T. S. Magnuson1, M. Swenson1, L. A. Deobald2; 1Idaho State Univ., Pocatello, ID, 2Univ. of Idaho, Moscow, ID
Abstract Body:
Background: Iron reduction and respiration is a common and utilitarian function in microbial systems in terrestrial, subsurface, and aquatic environments. Although there are many genera that are known or posited to be capable of this process, only a few have been studied in detail regarding the biochemical and physiologic mechanisms that enable this unique lifestyle. We have studied two model extreme Fe-reducing isolates in detail, and have found commonalities, as well as unique adaptations, for survival in habitats where Fe-respiration is advantageous. Methods: Extremophilic Fe-reducing bacteria (eFeRB) were cultivated with solid- or solution-phase Fe(III), under conditions (pH, T) that mimic their natural environment. Outer membrane vesicle and biofilm matrix proteins were isolated via differential chemical extraction followed by removal of whole cells. Protein fractions were analyzed by MS-enabled proteomics. Fluorescence microscopy was done using protein- and lipid-specific stains to examine microbe-mineral interactions and localization of biomolecules within vesicles and matrix material. Results: Under biofilm-growth conditions, Acidiphilium cryptum (pH 2, T 30°C) produces a suite of monoheme cytochromes c, and localizes these to the outer membrane, periplasm, and outer membrane vesicles where they are available to transfer electrons to solid phase minerals. Thermovenenabulum ‘Vulcan’ (pH 8, T 70°C) reduces Fe(III) in both solid and solution phase forms, and exports a variety of redox-active active proteins to the cell surface. Microscopy reveals significant attachment to minerals in both systems. Conclusions: The most significant comparisons reveal that acidophiles have redox proteins adapted to function in low pH environments, and are reliant on acid stable monoheme cytochromes for Fe-reduction. Thermoalkaliphiles appear to not rely on cytochromes, but rather other redox-active proteins (FeS, quinoproteins) for extracellular electron transfer. Both systems, however, produce a large proportion of proteins devoted to metal/nutrient transport and attachment/biofilm formation, suggesting that these functions are constant among ecotypes, and that redox protein functions vary in response to Fe-reducing conditions in situ. This study reveals that a common physiologic mechanism for iron reduction may not exist among extremophile eFeRB.
Biofilm Formation and Quorum Sensing in Marine Bacteria Isolated from Ballast Tanks

A. R. Malalasekara, D. P. Henderson, A. L. Oldham; Univ. of Texas of the Permian Basin, Odessa, TX

Abstract Body:
Biofilm formation in marine systems is associated with economic and ecological problems. Biofilms that form in the ballast tanks of Navy ships are one such example where microbial influenced corrosion can increase maintenance costs. These types of biofilms can also harbor invasive and pathogenic species, and in turn, seed other water bodies among global ecosystems. Since most of the conventional treatment approaches including antifouling coatings and biocides have detrimental effects on the environment, new sustainable strategies to limit biofilm formation are in demand. One such strategy is to target quorum sensing, which some bacteria use to regulate biofilm formation. In this study, bacterial isolates were recovered from ballast tank fluids and screened for the ability to form biofilms using crystal violet fixation. Out of the 22 isolates screened, 14 formed quantifiable biofilms. Six isolates formed biofilms in a synthetic sea water medium, two isolates formed biofilms in the same medium amended with tryptone and yeast extract, and six isolates formed biofilms in both types of media. These isolates were then screened for the potential to use quorum sensing using a degenerate set of primers that amplify luxI homologues of the autoinducer synthase gene. The amplification of a single gene product of the correct size was detected in at least three of the 22 isolates. While this screen is ongoing, these preliminary results suggest that biofilm formation may be associated with quorum sensing in at least three of these ballast tank isolates. These types of marine organisms might be useful in high-throughput quorum-sensing inhibitor studies.
Abstract Title:
Control of Carbapenem-Resistant Klebsiella Pneumoniae Biofilms Using A Nonionic Surfactant
Primary Author Block:
M. Mazher, A. J. Santiago, R. M. Donlan; CDC, Atlanta, GA
Abstract Body:
Introduction: Handwashing station sink drains are potential reservoirs for carbapenem-resistant Enterobacteriaceae (CRE), including carbapenemase-producing Klebsiella pneumoniae KPC+ (CRKP). CRKP have been shown to colonize sink drain P-traps and form biofilms, and are difficult to eradicate using traditional treatment strategies. The goal of this study was to identify a treatment strategy that could disperse CRKP biofilms without the use of disinfectants or biocides, which may be ineffective against biofilm organisms in these systems. Nonionic surfactants, such as polyoxyethylene-polyoxypropylene block copolymer surfactants (EO/PO BCS) can alter the surface tension at the biofilm/substratum interface and allow removal of biofilm-associated cells. We investigated the ability of P103, an EO/PO BCS to disperse biofilms of K. pneumoniae 1016 KPC+ (pKPC_UVA010) (K. pneumoniae 1016). Methods: For all experiments, K. pneumonia 1016 biofilms were grown in 96-well microtiter plates for 48 h at 37°C. Biofilms were exposed to a range of P103 concentrations (0.25 - 250 mg/L) for 0.5, 1, 3, or 24 h and treated 1, 2, or 3 times. Efficacy was determined by quantifying residual biofilm biomass using the crystal violet assay. Results: A single treatment of P103 was unable to reduce K. pneumoniae 1016 biofilm counts at all concentrations and all time points tested. However, three consecutive 30-min treatments of 0.25 or 0.5 mg/L reduced biofilms (p<0.05) by 20% and 13%, respectively. Three consecutive 1-h treatments of 0.25, 0.5, 1.0, and 2.0 mg/L reduced biofilms (p<0.05) by 24, 33, 21, and 18 %, respectively. P103 treatments greater than 2.0 mg/L were ineffective. Conclusions: P103 surfactant demonstrated dispersal activity against biofilm-associated K. pneumoniae 1016. Multiple treatments at lower use concentrations were significantly more effective. Future investigations will investigate the mechanism for P103 effectiveness against established biofilms and evaluate a treatment strategy incorporating P103 to control CRKP in native polymicrobial biofilms of sink drain P-traps in handwashing sinks of healthcare facilities.
Abstract Title:
Characterization of Surface Modified Orthopedic Implants for their Antimicrobial Properties

Primary Author Block:
S. Beladi Behbahani, S. Helms, J. Desjardins, M. Kennedy, T. Bruce, J. Tzeng; Clemson Univ., Clemson, SC

Abstract Body:
Contamination of combat trauma wounds with environmental residues can lead to bacterial infection of orthopedic fractures, which causes delay and difficulties in patient treatment. The reported infection rate of injuries to U.S. troops in the period of 2003 to 2007 from improvised explosive devices (IED) was reported as 91%, and biofilm formation on orthopedic implants can lead to chronic infection with a rate of 40% in fracture wounds. Once the biofilm has formed, it will become resistant to antibiotics. Therefore, this study focused on designing orthopedic implants that could self-regulate local infection and biofilm formation. Polytetrafluoroethylene (PTFE) and biodegradable chitosan with local antibiotic (vancomycin) elution were deposited onto coupons of stainless steel and titanium alloys which are utilized as implant materials. The study looked at the response of Staphylococcus aureus, which is the most common pathogen associated with orthopedic implant infections. The response of the S. aureus Seattle 1945 (ATCC 25923) strain encoding intracellular GFP was evaluated utilizing crystal violet analysis, ultrasound water bath with viable cell counts and confocal laser scanning microscopy. The release rate of vancomycin from the coupons was also monitored through HPLC analysis of collected leachates from surface modified coupons. In vitro studies of antibacterial properties of the coupons showed that coupons with PTFE did not provide significant advantages against biofilm formation. However, coupons which were coated with chitosan and vancomycin prevented biofilm formation during the in vitro studies. LCSM scanning of the modified surfaces with vancomycin did not indicate the detection of any GFP signal. In addition, no bacterial cells were recovered from the vancomycin treated coupon surfaces. Local drug-release profile of antibiotic doped chitosan showed the concentration of local vancomycin released within the first 48 hours was effective in preventing bacterial attachment onto the coupons. Based on data obtained from these in vitro studies, it is concluded that vancomycin treated coupons were able to successfully prevent biofilm formation and bacterial growth on the modified surfaces.
Abstract Title:
Analysis of Biofilm Production between Microbacterium and Chryseobacterium Sp

Primary Author Block:
E. Vickers, A. Finck, N. Sy, S. DiDomenico, Z. Whatley; Gettysburg Coll., Gettysburg, PA

Abstract Body:
Biofilms that occur naturally in aquatic systems can pose a threat to human health. In hospital water systems, aquatic biofilms can promote the presence of pathogenic strains such as Legionella pneumophila, and this can lead to nosocomial infections in patients. Previous studies have recovered Chryseobacterium and Microbacterium species from biofilms at various stages of water treatment systems. Understanding the interaction between these non-pathogenic bacteria and their role in sustaining microbial communities for pathogenic bacteria could advance water treatment systems. We report enhanced biofilm formation between Microbacterium and Chryseobacterium isolates from a drinking water system. The objective of this study is to explore the genetic basis of the interaction between these isolates. Preliminary results suggest that the synergism is driven by cell surface interactions, leading us to focus on the role of extracellular proteins and polysaccharides, naturally occurring plasmids, and the Type VI and IX Secretion Systems. The T9SS has been linked to gliding motility, virulence, adhesion, and biofilm formation in other members of the Cytophaga-Flavobacterium-Bacteroides group. We employ genetic techniques, including the generation & screening of transposon mutants and heterologous expression libraries, to further characterize the Microbacterium-Chryseobacterium interaction.
Abstract Title:
Ease of Biofilm Accumulation & Efficacy of Sanitizing Treatments in Removing the Biofilms Formed, on Selected Abiotic Surfaces

Primary Author Block:
H. Gazula, J. Chen; Univ. of Georgia, Griffin, GA

Abstract Body:
Background: Biofilms formed by bacteria on food contact surfaces can increase the risks of product contamination. This study assessed 1) the formation of biofilms by fecal coliforms, isolated from different blueberry packing lines, on selected abiotic surfaces and 2) the efficacy of different sanitizing treatments in removing the biofilms. Methods: Biofilm-forming ability of six bi-strain mixtures of fecal coliforms was assessed at 10°C for 7 days on coupons made of materials commonly found on blueberry packing lines including high-density polyethylene, stainless steel, rubber, polyvinyl chloride, polypropylene, polyurethane, and recycled polyethylene. Surface coupons with developed biofilms were treated for 1 min with sanitizers used by blueberry packers including 5 ppm active chlorine dioxide, 3 ppm ozonated water, 200 ppm quaternary ammonium, or 200 ppm sodium hypochlorite. Residual biofilms on treated coupons were quantified using the crystal violet binding assay. Results: The amount of biofilms accumulated on polypropylene were significantly higher (p<0.05) than on polyurethane coupons. Biofilms formed on polyvinyl chloride and rubber coupons were statistically similar, but were significantly lower than those on polypropylene and polyurethane coupons and significantly higher than those on high-density polyethylene coupons. Recycled polyethylene and stainless steel coupons had similar amounts of biofilm mass, but were significantly lower than those on other coupons. Ozonated water had significantly higher efficiency in biofilm removal than quaternary ammonium compound which had significantly higher efficiency than chlorine dioxide, followed by sodium hypochlorite. Significantly more residual biofilm mass was found on rubber coupons. Residual biofilm mass on polypropylene, polyvinyl chloride, and high-density polyethylene coupons was statistically similar, but they were significantly higher than those on polyurethane and stainless steel coupons. Conclusion: The evaluated sanitizers had different efficacies in biofilm control. The type of coupons and fecal coliform isolates involved in the study played a significant role in biofilm accumulation and removal on the selected abiotic surfaces.
Biofilm Formation by Marine Bacteria, Isolated from Coastal Waters of Karachi Pakistan

Primary Author Block:
A. Shaheen1, S. U. Kazmi2, H. S. Baig1; 1Natl. Inst. of Oceanography, Karachi, Pakistan, 2Dadabhoy Inst. of Higher Ed., Karachi, Pakistan

Abstract Body:
Biofilm formed by bacteria plays a vital role in marine environment to transfer metal/minerals from sedimentary environment to bacteria. In order to determine whether the adherence abilities to marine bacteria living in different environmental scenario, differed them individually from others, quantifications of cell adhesion and biofilm formation by single isolates was performed. For qualitative detection of biofilm formation by marine isolates was studied by test tube biofilm assay. To quantify the biofilm formation by marine isolates microtitre plate biofilm assay was performed. Total 197 different marine bacteria were isolated and identified from marine environmental samples collected from coastal waters of Karachi, Pakistan. They included 70 (35.53 %) free bacterial strains isolated from sea water and 127 (64.47 %) attached bacterial strains isolated from mud and rock surfaces. 118 (60%) out of 197 total isolated marine bacteria were adherent and showed clear visibility of adherence to the surfaces of test tube. Isolated attached bacteria were more fairly adherent than free bacteria with total percentage assemblage of adherent bacteria with 67% and 33% respectively. In order to quantify the biofilm, marine bacteria were screened in 96 well microtitre plate biofilm assay at different incubation period and was quantified by crystal violet staining method. According to the optical density measured at 570 nm wavelength after 48 hrs incubation, screened marine bacteria were classified into three groups, group I: (OD570 ≥ 1), 78 strains out of 197 (40%) were significantly stronger biofilm forming, group II: (0.1 ≤ OD570 < 1) 40 (20%) strains were low grade/ weaker biofilm forming, and group III: (OD570 < 0.1), 79 (40%) were negative/non-biofilm forming bacteria. Furthermore, we noticed that level of biofilm formation was significantly increased (approximately doubled) from 3 to 48 hours. Three hours incubation periods was the initiation period when marine bacteria start to grow after log phase and 48 hours incubation period was the time period where the bacteria almost far more than doubled of initial bacterial cells. These results clearly indicate the function of biofilm formation in marine bacteria. Higher sedimentary attached bacteria showed that such surfaces plays major role in transformation of nutrients to the bacteria for their growth even under such stressed conditions.
Abstract Title:
Influence of Type I Fimbriae and Fluid Shear Stress on Bacterial Behavior and Multicellular Architecture of Early Escherichia coli Biofilms on Polyethylene Terephthalate (Pet) Surfaces

Primary Author Block:
L. Wang1, Q. Zhao1, C. Bryant2, E. Terentjev2;  1Univ. of dundee, Dundee, United Kingdom, 2Univ. of Cambridge, Cambridge, United Kingdom

Abstract Body:
Background Biofilms are usually studied at a rough macroscopic level; thus, how biological and physical factors determine cellular architecture of early biofilms and behavior of constituent cells remains largely unknown. In this study, we examined the specific role of type I fimbriae in nascent E. coli biofilms and the response of microcolonies to fluid shear at a single-cell resolution. Methods The E. coli MG1655 ΔfimA::kan mutant strain was constructed as described previously1. Clean PET surfaces were incubated with wild-type or mutant E.coli suspension for 1 h-adhesion, before external LB medium was hourly refreshed. At each hour interval, before and after treatment of shear stress (0.6, 1.4 and 3.3 Pa, labelled as low, medium and high stress, respectively), the stained E.coli cells on surfaces were observed by confocal microscopy. To study the effect of 3D architecture on the response of biofilms to fluid shear, we incubated seeded PET with hourly refreshed LB containing 1.56 mg/L Furanone C-30 (FC30, a quorum sensing inhibitor as signal antagonist2). Bacterial growth on surfaces was not altered but 3D structure was changed into single-layered colonies3. Results Type I fimbriae are not required for reversible adhesion from plankton but are critical for the irreversible adhesion of E.coli on surfaces. The irreversible adhesion seems necessary to initiate the proliferation of E. coli on the surface (Figs. 1-2). After the application of shear stress, bacterial retention is dominated by 3D architecture of colonies independent of the population size, and the multi-layered structure could protect the embedded cells from being insulted by fluid shear, while the cell membrane permeability mainly depends on the biofilm population size and the duration of the shear stress (Figs. 3-6). Conclusion After adherent bacteria start to grow into microcolonies, detachment is difficult. Fluid shear could be combined with cell signalling inhibitor to remove bacteria from surfaces.<p><a href="http://files.abstractsonline.com/CTRL/4d/c/9e3/0dd/8ee/4c5/080/857/2a9/a1c/a56/59/g5733_1.jpg" target='_blank' address=no /><img src="http://files.abstractsonline.com/CTRL/4d/c/9e3/0dd/8ee/4c5/080/857/2a9/a1c/a56/59/g5733_1.jpg" alt="" border="0" width="522" height="792" height="792" width="522" /></a></p>
Abstract Title:
Aggregation of Staphylococcus aureus And Escherichia coli In Synovial Fluid Causes Antimicrobial Tolerance Due Changes in Growth Kinetics, Atp Production and Cellular Respiration

Primary Author Block:
J. Gilbertie; North Carolina State Universirt, Raleigh, NC

Abstract Body:
Infectious arthritis is a medical emergency and treatment of this condition can be prolonged and unrewarding. Bacterial grown in synovial fluid (SynF) form macroscopic aggregates, similar to biofilms, which display severe antimicrobial tolerance. In order to investigate the cause of antimicrobial tolerance in SynF aggregates, Staphylococcus aureus and Escherichia coli were inoculated into SynF (n=6) or TSB (control, n=6) at 1x10^6 CFU/mL and incubated at 37°C. A growth curve was generated over 96 hours. Over the first hours, SynF bacteria decreased ~2log compared to an increase in TSB (p<0.0001). SynF bacteria grew significantly slower and reached a lower concentration then TSB at 24 hours (p<0.0001). A delayed death phase in SynF (72 hours vs 48 hours for TSB) was also observed. Thereafter, bacteria were grown to exponential phase (4 hours for TSB and 8 hours for SynF) and ATP and respiration were measured using bioluminescent and fluorescent reporter assays. Bacteria within SynF had minimal ATP production compared to TSB grown bacteria (p<0.0001). Likewise, bacteria within SynF displayed decreased respiration (p<0.0001). Proteinase K, previously shown to disperse SynF bacterial aggregated and restore antimicrobial efficacy, was added to infected SynF for 15 minutes prior to measuring ATP and respiration. When aggregates were dispersed there was increased ATP production (p<0.0001) and increased respiration (p<0.0001). The changes in the bacterial growth kinetics, production of ATP, and respiration indicate a dormant state of SynF aggregated bacteria. As most antimicrobials act on actively growing bacteria, we concluded that the antimicrobial tolerance observed in bacteria aggregated within SynF could be due to decreased cellular processes and energy generation by the electron transport chain. Further experiments are required in order to validate this speculation.
Abstract Title:
Antibiofilm Efficacy of Peptide against Listeria Monocytogenes and Escherichia coli O157:H7 on Equipment Surfaces

Primary Author Block:
A. Boomer1, H-B. Yin2, J. Patel3; 1Charles Herbert Flowers High Sch., Springdale, MD, 2Univ. of Maryland, Beltsville, MD, 3USDA, Beltsville, MD

Abstract Body:
Background: Foodborne bacterial pathogens Listeria monocytogenes and Escherichia coli O157:H7 have ability to colonize and form biofilms on a wide range of equipment surfaces at the food processing facilities. Pathogens in biofilms are resistant to conventional antimicrobials and require higher antimicrobial concentrations to inactivate biofilms. There is a need to identify compounds that effectively inactivate biofilms on equipment surfaces to prevent cross-contamination and protect public health. In this study, we investigated the efficacy of a synthetic innate defense regulator peptide 1018 (IDR-1018) for inactivating L. monocytogenes and E. coli O157:H7 biofilms on stainless steel and polycarbonate surfaces. Methods: For biofilm formation, stainless steel and polycarbonate coupons (n=96) were used in the CDC-biofilm reactor containing 400 ml of 10% trypticase soy broth (TSB), that had been inoculated with L. monocytogenes or E. coli O157:H7 to obtain 6 log CFU/ml populations. The reactor was set with a constant flow rate at 50 ml/h of 10% TSB for 48 h. After 48 h, coupons were treated with IDR-1018 at 0, 10, 20, or 50 µg/ml concentrations in phosphate buffer saline (PBS) for 24 h. Surviving bacterial populations were determined by scrapping off the coupons and spiral plating on selective media. Results: Significantly higher levels of L. monocytogenes and E. coli O157:H7 biofilms were recovered on polycarbonate surfaces (~ 6.5 log CFU/cm²) than on stainless steel surfaces (~5 log CFU/cm²). Antibiofilm efficacy of IDR-1018 against pathogens was concentration-dependent and varied with the type of pathogen and material surfaces. L. monocytogenes was more sensitive to IDR-1018 than E. coli O157:H7. L. monocytogenes was reduced by 2-4.2 log CFU/cm² following treatment with 10-50 µg/ml IDR-1018 compared to 1.5-2.5 log CFU/cm² reduction with E. coli O157:H7 on stainless steel. The IDR-1018 exerted higher antibiofilm ability against L. monocytogenes on stainless steel surface than on polycarbonate surface. At 50 µg/ml conc., it significantly inactivated L. monocytogenes biofilms by 4.2 and 3.0 log CFU/cm² on stainless steel surfaces and polycarbonate surfaces, respectively (P<0.05). Conclusion: Result suggest that IDR-1018 may be used to inactivate L. monocytogenes and E. coli O157:H7 biofilms on equipment surfaces.
Abstract Title:
Bacterial Growth and Cell Size Control on Polyethylene Terephthalate (PET) Surfaces At Single-Cell Resolution

Primary Author Block:
L. Wang1, Q. Zhao1, E. Terentjev2; 1Univ. of Dundee, Dundee, United Kingdom, 2Univ. of Cambridge, Cambridge, United Kingdom

Abstract Body:
Background: Bacterial biofilms can cause severe infections in clinical settings, yet little is known about dynamics and mechanisms of the multicellular architecture and the individual bacterium behavior during early biofilm development on solid substrates. In this study, we applied confocal laser scanning microscopy (CLSM) to investigate the adhesion, the growth and the cell size control of Escherichia coli (E. coli) MG1655 on PET surfaces at a single-cell resolution. Methods: Figure 1 illustrates the overall procedure. Briefly, clean substrates were incubated with E.coli suspension for 1 h-adhesion, before LB medium incubating these seeded PET surfaces was hourly refreshed. At each hour interval, the amount of stained E.coli on surfaces was counted from CLSM images using Image J, and the number of cells detaching from surfaces into medium was measured by agar plate counting. To access the role of quorum sensing in architecture and bacterial size control in biofilms, we incubated the seeded PET samples with hourly refreshed LB containing 1.56 mg/L Furanone C-30 (FC30, a quorum sensing inhibitor as signal antagonist). At this low concentration, bacterial growth rate on substrates was not altered (Fig.2). Results: Figures 3-4 show that the bacterial growth curve on PET exhibits the distinct lag and log phases, but the generation time (38min) is more than twice longer than in bulk medium (16 min), which is not due to the detachment of daughter cells as the detachment is quite low (Fig.5). When the quorum sensing is inhibited, the 3D structure of biofilms disappears, and the bacteria in clusters retain their large size as they have as singles up to 9 h of growth on surfaces, while with no inhibition - the size rapidly decrease started between 6 and 7 h (Figs. 6-7). Conclusion: The cell size is under the density-dependent pathway control: when the clustered cells are at high density, quorum sensing causes the cell size decrease as the cell density on surfaces increases (Fig.8).
Abstract Title:
Impact of Staphylococcus Epidermidis Secreted Molecules on Biofilm Production of Staphylococcus Spp. Isolated from Endocarditis

Primary Author Block:
J. C. M. Campos1, T. Glatthardt1, R. C. Chamon1, L. M. Antunes2, K. R. N. Dos Santos1, R. B. R. Ferreira1; 1Federal Univ. of Rio de Janeiro, Rio de Janeiro, Brazil, 2Fiocruz, Rio de Janeiro, Brazil

Abstract Body:
Several infections are caused by Staphylococcus spp., among them infective endocarditis (IE), which has a high mortality rate. Two factors associated with the pathogenesis of these bacteria are the high antimicrobial resistance and the ability to form biofilm, which also confers protection against antibiotics and the immune system. Therefore, there is a demand for new therapeutic options against these pathogens. Staphylococcus spp. are also found predominantly in the skin microbiota, and it was already reported that S. epidermidis can secrete compounds that inhibit colonization by pathogens. The aim of this study was to evaluate the impact of molecules secreted by S. epidermidis on growth and biofilm production of Staphylococcus spp. isolates from IE. The supernatant of commensal S. epidermidis was obtained, filtered and concentrated and its effect evaluated on growth and biofilm production of the clinical isolates of Staphylococcus spp. previously identified (8 S. aureus, 5 S. epidermidis, 4 S. haemolyticus and 4 S. hominis). Among the 21 isolates, 12 (57.1%) were biofilm-producers, including 7 S. aureus, 2 S. epidermidis, 2 S. haemolyticus and 1 S. hominis. A negative impact on that production was observed in 10 (83.3%) isolates when they were grown in the presence of the supernatant, without causing any effect on growth. Thus, among the biofilm-producers, 7 (100%) S. aureus, 1 (50%) S. epidermidis, 1 (50%) S. haemolyticus and 1 (110%) S. hominis exhibited less biofilm in the presence of commensal S. epidermidis secreted molecules. Biofilm composition was determined for these isolates and all presented a mainly protein-based biofilm. Typing of the agr operon, an important virulence regulator, revealed that most of these isolates were type I (37.5%) and type III (50%). We also investigated for the presence of genes related to biofilm production (ica, sasG and aap) by PCR. Seven (70%) isolates carried the ica gene, all S. aureus had the sasG gene, and the S. epidermidis was positive for the aap gene. The results indicate that some Staphylococcus spp. isolates from IE can produce biofilm at the conditions used and that S. epidermidis secreted molecules have activity against the biofilm produced, suggesting a promising therapeutic potential of this extract.
Abstract Title:
Effects of Nano-Hydroxyapatite and Nano-Zinc Oxide on the Formation and Removal of Biofilms Produced by Streptococcus Mutans

Primary Author Block:
M. Park, F. Rafii, J. B. Sutherland; Natl. Ctr. for Toxicological Res., jefferson, AR

Abstract Body:
Background: Dental caries is caused by the growth of clusters of bacteria after the initial formation of biofilm on a tooth surface. The use of nanomaterials to combat biofilm formation is being considered for new applications in dentistry. Streptococcus mutans is actively involved in the first step of biofilm formation through production of glucans, which results in the bacterial cells adhering to the tooth surface. This species is a major contributor to the development of biofilms and its control is important in the prevention of dental caries. Methods: We have examined the effect of nano-hydroxyapatite (20-50 nm) and nano-zinc oxide (40-100 nm) on the growth, adhesion and removal of biofilms produced by S. mutans in 96-well plates and on a hydroxyapatite-coated surface representing the major structural component of tooth enamel. The kinetics of growth of S. mutans in the presence of different concentrations of nanomaterials were monitored spectrophotometrically. The effects of nano-hydroxyapatite and nano-zinc oxide on cell growth and adherence, and on the disruption of biofilms formed, were measured by neutral red staining. Results: Increasing the concentrations of nano-hydroxyapatite and nano-zinc oxide decreased the rate of growth of S. mutans. Nano-zinc oxide, at a concentration of 5%, inhibited cell growth both in the presence and the absence of sucrose. Increasing the concentration of nano-hydroxyapatite decreased cell adherence, in comparison with the cultures without nano-hydroxyapatite, both in the absence and the presence of sucrose. A 2.5% concentration of nano-zinc oxide decreased cell adherence only in the presence of 5% sucrose. Neither nano-zinc oxide nor nano-hydroxyapatite removed biofilms that had formed on the hydroxyapatite-covered surfaces before treatment with nanomaterials. Conclusions: The results of these in vitro experiments show that nano-hydroxyapatite and nano-zinc oxide may be useful in reducing growth of S. mutans and may inhibit bacterial attachment to surfaces, but even at high concentrations they do not reduce the formation of biofilms after cell adherence.
Abstract Title:
Outstanding Abstract Award: Growth and Biofilm Formation of Oral Bacteria after Exposure to Electronic Cigarette-Generated Aerosol and Conventional Cigarette Smoke

Primary Author Block:

Abstract Body:
Background: The oral cavity is covered by bacteria, mostly commensal streptococci, on all surfaces including mucosal epithelia and teeth. Because of their commensal nature and as an effort to maintain the oral environment at homeostasis, it is believed that these oral bacteria act as a layer of protection from the external milieu. External challenges, in the case of humans, include smoking conventional cigarettes or vaping the “harm-reduction” alternative, electronic cigarettes (ECIGs). Therefore, we tested the effects of ECIG-generated aerosol and conventional cigarette smoke on the growth and survival of four oral streptococci using a unique exposure system designed to closely emulate the effects of aerosol or smoke in the oral cavity. Methods: Briefly, peristaltic pumps were used to transfer aerosol or smoke into chambers containing bacterial samples. The puff protocol consisted of 25, 50 or 75 cycles of a five-second puff (pumps on) followed by a ten-second rest period (pumps off). Then, bacteria were immediately incubated for growth at 37°C, 5% CO2 and results were analyzed the following day. Results: After exposures up to 75 puffs of ECIG-generated aerosol, our results indicate that the four species of oral streptococci appear to grow normally. However, 25 to 50 puffs of cigarette smoke were enough to kill most or all of the four streptococci tested. Based on these results we are investigating the effects of ECIG-generated aerosol and cigarette smoke on biofilm formation of all four oral species. We hypothesize that ECIG-generated aerosol has, at most, a modest effect on biofilm formation and architecture but smoke will either kill all bacteria or elicit a radical impact on biofilm formation and/or architecture. Conclusions: To our knowledge, our project is the first to address the influence of ECIG-generated aerosol on oral bacteria as compared to that of cigarette smoke. Our results indicate that, compared to smoking, use of ECIGs is not as detrimental to the survival of commensal oral bacteria. Since the bacteria remain alive post-exposure to ECIG-generated aerosol, they may continue to function as a protective layer above the human oral tissue, further supporting ECIGs as a “harm-reduction” alternative to conventional cigarettes.
Abstract Title:
Rhamnus Prinoides (Gesho): A Source of Diverse Antimicrobial and Anti-Biofilm Activity

Primary Author Block:
M. Campbell, E. Gilbert; Georgia State Univ., Atlanta, GA

Abstract Body:
Biofilm infections are a major source of chronic illness and have negative impacts on disease severity and patient prognosis (1). Biofilm infections are exceptionally difficult to treat due to their facilitation of antibiotic resistance (2). In recent years, research into anti-virulence compounds has increased in an effort to develop effective anti-biofilm therapeutics that do not perpetuate resistance. Traditional medicine may provide a source for such compounds. Rhamnus prinoides (gesho), an evergreen shrub from East Africa, has been traditionally used for the treatment of various illnesses including atopic dermatitis (3). We hypothesized that gesho extracts could be a source of anti-biofilm compounds effective against Gram positive bacteria. Lyophilized ethanolic and aqueous extracts were prepared from dried gesho stems and leaves. Biofilm inhibition was measured via crystal violet staining and subsequent viability assays were conducted on growth agar. Rhamnus prinoides leaf ethanol extracts significantly prevented Staphylococcus aureus, Bacillus subtilis and Streptococcus mutans biofilm formation up to 99 percent relative to untreated controls. Chromatography, chemical tests, Fourier transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GC-MS) were used to isolate and identify active compounds. Activity screens identified ketone and benzoic compounds with anti-biofilm activity. Our work suggests gesho leaf ethanol extracts contain a diverse set of chemicals with anti-biofilm activity. This work lends support to the traditional use of gesho for the treatment of biofilm associated topical infections and warrants further investigation into Rhamnus prinoides as a source of anti-biofilm compounds.
Antibiofilm Activity of Staphylococcus Epidermidis Secreted Molecules against Staphylococcus aureus

Primary Author Block:
T. Glatthardt1, J. C. M. Campos1, T. F. S. Coimbra1, R. C. Chamon1, L. M. Antunes2, K. R. N. Dos Santos1, R. B. R. Ferreira1; 1Federal Univ. of Rio de Janeiro, Rio de Janeiro, Brazil, 2Fiocruz, Rio de Janeiro, Brazil

Abstract Body:
The skin is colonized by a wide range of microorganisms and S. epidermidis is one of the most frequently found species in this microbial community. S. epidermidis can limit the growth of some pathogens by producing proteases and bacteriocins, and this includes skin pathogens such as S. aureus. S. aureus causes diverse types of infections, ranging from skin abscesses to life-threatening bloodstream infections. Resistance to several antibiotics is a common virulence trait of S. aureus, and methicillin-resistant isolates have been a major public health concern due to the limited treatment options. Given the increasing prevalence of resistant strains, it is imperative to search for new strategies against this pathogen, and the use of anti-virulence compounds has been raised as an innovative approach. Thus, this study aimed to investigate the impact of the molecules secreted by commensal S. epidermidis on virulence traits of clinical S. aureus strains. S. epidermidis isolated from skin microbiota was grown to stationary phase and the bacterial supernatant was collected, filtered and concentrated. We first analyzed the impact of the concentrated supernatant on global transcription by RNAseq on biofilm cells of S. aureus, since this is an important virulence state of these strains. A significant repression of genes related to biofilm formation was observed when S. aureus biofilm was grown in the presence of the S. epidermidis supernatant. In fact, by using a microplate biofilm assay, we showed that the molecules present in the supernatant of S. epidermidis caused a reduction in biofilm production in 65.5% S. aureus clinical strains tested. We also observed that the activity on biofilm formation was independent from bacterial planktonic growth. S. epidermidis supernatant also exhibited a significant impact on preformed S. aureus biofilm, showing a dispersion effect. We were able to show that such activity could be potentiated by using with sub-minimal inhibitory concentrations of oxacillin.

Preliminary characterization results showed that the active compound have a molecular weight between 3kDa and 10kDa, is resistant to proteinase K and boiling for 40 minutes, as well as is soluble in ethyl acetate, suggesting a rather lipidic nature. Biofilm formation is one of the main virulence factors of S. aureus and has been related to chronic and recurrent infections and antimicrobial resistance. Therefore, molecules that can counteract this virulence factor could lead to the discovery of new therapeutic agents for the control of S. aureus infections.
Abstract Title:
Biofilm Formation and Motility Play a Role During Interactions of Salmonella Typhimurium with Acanthamoeba Castellani

Primary Author Block:
I. Ahmad, Muhammad Wasim, Talha Manan, Arsalan Gil, Ute Romling, Abdul Matin; Univ. of Hlth.Sci., Lahore, Lahore, Pakistan

Abstract Body:
Background: Pathogenic bacteria share natural habitat with many other organisms such as animals, plants, insects and parasites. These interactions influence life style of not only host organisms but also modulate bacterial life style. Modulations in bacterial life style include but not restricted to biofilm formation, capsule formation and secretion of virulence factors to penetrate host tissue. In this study, we investigated the role of molecular pathways mediating rdar biofilm formation, type 1 fimbriae biogenesis, chemotaxis and motility systems in the interaction process of Salmonella typhimurium with Acanthamoeba castellanii T4 genotype. Since both organisms share natural habitat therefore, such an interaction is both clinical and environmental relevant phenomenon. Methods: we created Salmonella typhimurium strains lacking the capability to express one or more components of rdar biofilm formation by deleting genes encoding rdar biofilm master regulator CsgD, cellulose synthase BcsA, curli fimbriae subunits CsgBA, type 1 fimbriae subunit FimA, type 1 fimbriae tip protein FimH, Flagella subunits FlIC/FljB and chemotaxis system proteins CheY and CheA using the λ Red recombination system. We tested wild type S. typhimurium and mutant strains for capability to associate with, invade into and survive inside Acanthamoeba castellani T4.<u></u><strike></strike> Results: Assessment of wild type S. typhimurium and mutants for capability to associate with, invade into and survive inside Acanthamoeba castellani T4.<u></u><strike></strike> Conclusions: Our findings suggest that cellulose an important component of S. typhimurium biofilm protects the bacteria from taking up by Acanthamoeba whereas type 1 fimbriae and flagella promote such interactions.
**Session Number:** 77  
**Session Type:** Poster

**Session Title:** AES04 - Biofilms in Applied and Environmental Science: Biofilm - Slime that Helps or Hurt

**Session Start Date Time:** 6/8/2018 11:00:00 AM  
**Session End Date Time:** 6/8/2018 1:00:00 PM

**Session Primary Track:** Applied and Environmental Science  
**Abstract Control Number:** 4417  
**Poster Board Number:** FRIDAY - 821

**Abstract Title:**  
Straight from the Tap: A Real-Time Biofilm Model for Med. Devices Containing Non-Sterile Water

**Primary Author Block:**  
D. Wolloscheck, A. Garg, V. M. Hitchins, J. W. Weeks; US Food and Drug Admin., Silver Spring, MD

**Abstract Body:**  
**Background:** Heater-Cooler Devices (HCDs) are important medical devices for patient thermoregulation during cardiothoracic surgeries. Manufacturer’s Instructions for Use (MIFU) recommend the use of sterilized water for reprocessing and filling of tanks. However, it is oftentimes common practice to use tap water to fill HCDs, leading to the development of biofilms inside these devices. Biofilms are known for their resistance to cleaning and disinfection, potentially leading to elevated bioburden inside HCD. Nontuberculous Mycobacteria (NTM) are emerging pathogens commonly found in tap water. Recently, M. chimaera and other NTMs have linked HCD biofilms to patient infections. Furthermore, the presence of NTMs is difficult to detect due to fast-growing contaminating organisms.

**Methods:** Biofilms of NTMs were grown in sterilized tap water on stainless-steel coupons (SSC) to mimic the water tanks of HCDs. As per MIFU, water was changed weekly. After a month, biofilm was removed from the SSC and bacteria were enumerated. Dilute hydrogen peroxide was used to determine the effects of water treatment on biofilm formation. Additionally, chemicals were tested to remove fast-growing organisms without inhibiting NTM recovery. Finally, various chemicals were analyzed for their ability to reduce all bioburden after biofilms were established. Results: NTMs can form robust biofilms in tap water. In the presence of hydrogen peroxide, the biofilm associated NTM counts were significantly reduced (p <0.001). Cetylpyridinium chloride, a chemical regularly used for decontamination, also negatively affected NTM counts, suggesting it might be less suitable for quantitation of NTM in mixed populations. Conversely, sodium hydroxide based decontamination solutions showed only a marginal impact on NTM viability. When disinfectants were compared for their activity against tap water-grown NTM biofilm on SSC, sodium hypochlorite was found to be less effective than peracetic acid based solutions.

**Conclusions:** NTMs are resilient organisms, that our work demonstrates it can form biofilms in tap water on SSC. In this study, we investigated the effects of chemicals used for water treatment, decontamination, and disinfection of NTM biofilms. Water treatment with hydrogen peroxide reduced bioburden on the SSC and this effect was dependent on the species. In addition, sodium hydroxide based solutions are more adequate decontaminants to quantify NTMs. Lastly, peracetic acid based solutions are more effective at reducing NTM bioburden from SSC.
Abstract Title:
Characterization of Ship Microbiomes and Port Microbial Communities in the Great Lakes

Primary Author Block:
S. M. Techtmann, R. B. Ghannam, L. G. Schaerer, T. M. Butler, M. Breneman; Michigan Tech Univ., Houghton, MI

Abstract Body:
Ports throughout the world have been impacted by anthropogenic activity and invasive species. These locations are constantly being exposed to new inputs through ship traffic, industrial activity, and ballast water exchange. Ships carry with them a diverse microbial community. The microbiome of a vessel may to some extent reflect the waters through which it has passed. Many ports undergo dramatic seasonal changes, that can alter the composition of the microbial communities in these ports. We investigated the impact of seasonal change and ship traffic on the microbial community composition of three ports in the Great Lakes. Samples were collected from Duluth-Superior, Green Bay, and the Keweenaw Peninsula in Fall of 2016 and Summer of 2017. Samples were also collected from the boats used for sampling to identify the microbiome of the vessels and how the waters through which these vessels transits impact the vessel microbiome. The microbial community composition of these samples was profiled using 16S rRNA sequencing. The environmental conditions in these ports changed marginally between these two sampling times with temperature changing the most substantially between sampling events. The overall microbial community composition was significantly different between these three ports based on PERMANOVA analysis. Ports on the same body of water had distinct microbial communities. Certain microbial groups are substantially enriched in particular ports relative to others. The microbial community composition changes dramatically in these ports between seasons. Bilge water and ship surfaces were also sampled to investigate the impact of ship traffic in the dispersal of microbes from one location to another. Our results also suggest that boats can carry indicator species from one port to others. For example, Microcystis was present at an average of 8.6% of the microbial community in Green Bay, and less than 0.01% of the community in the other ports. Bilge water was sampled from a boat that was in Green Bay and transported to Duluth. After sampling in Duluth, Microcystis were present at more than 30 times the abundance in Duluth waters. This indicates that microbes can be transported from one location to another via boat traffic, suggesting that ship traffic has the potential to contribute to microbial dispersal in aquatic environments.
Abstract Title:
Sub-Minimal Inhibitory Concentrations of Commonly Used Livestock Antibiotics Increase Biofilm Formation of Streptococcus Suis

Primary Author Block:

Abstract Body:
Streptococcus suis is a swine pathogen responsible for significant economic losses to the swine industry worldwide. S. suis is typically carried in the tonsil and nasal cavity of swine and is capable of causing a spectrum of infection outcomes ranging from asymptomatic carriage to lethal systemic disease. A key barrier towards the development of improved vaccines or interventions for S. suis infections is a gap in our understanding of the mechanisms contributing to persistence or the carrier state, in which colonized pigs continue to shed and transmit S. suis. Routine management practices involve treating all pigs in the same pen or barn with appropriate antibiotics upon observing any pig exhibiting clinical signs of S. suis-associated disease. This practice increases the probability of exposure of bacteria to sub-minimal inhibitory concentrations (sub-MICs) of antibiotics. Sub-MICs of antibiotics can alter bacterial gene expression and subsequent phenotype which can impact and/or alter the ability of bacteria to colonize, cause disease, and persist within the respiratory tract. We hypothesized that exposure to sub-MICs of antibiotics commonly used by the swine industry would increase the biofilm capacity of S. suis strains. Using a 96-well plate MIC protocol, we experimentally determined the MIC for each antibiotic for S. suis strain ISU1606, a virulent strain that consistently formed biofilms using a standard crystal violet assay. After verification that the sub-MICs do not affect kinetic growth, we tested the effect of sub-MICs on biofilm formation. Sub-MICs of amoxicillin, lincomycin, or oxytetracycline induced a statistically significant increase in biofilm formation. Antibiotic concentrations that exhibited an increase in static biofilm formation were additionally evaluated using a flow-cell biofilm assay, which demonstrated similar significant increases in biofilm formation. Collectively, our data expand the knowledge regarding S. suis biofilm formation and demonstrate that exposure to sub-MICs contributes to increase biofilm formation of S. suis, thereby potentially contributing to persistence or the carrier state.
Abstract Title:
Analysis of Ahls Level and their Function in Aerobic Sludge Granulation Under Different Srt

Primary Author Block:
L. Zhu, Z. Zhang; Zhejiang Univ., Hangzhou, China

Abstract Body:
Background: Aerobic granular sludge is a promising technology for its abundant biomass, excellent settleability and high pollutant removal efficiency, and various microbes with different growth characteristics and functions are enriched in granular sludge. Methods: In this study, the physical property and pollutant removal in aerobic granular sludge reactor were investigated under condition of different sludge retention time (SRT), and variation of acylated homoserine lactones (AHLs) was analyzed for the possible mechanism of sludge granulation. Results: Results showed that the aerobic granular sludge were formed in all the reactors operated at different SRT, and the percentages of granules with size over 200 μm were 13.12%, 19.54% and 10.01% in R1(SRT uncontrolled), R2(SRT of 6 days) and R3(SRT of 12 days), respectively. Thereinto, higher TN removal efficiency and lower SVI5 were achieved in R2 reactor. Statistical analysis for different kinds of AHLs indicated that most of detected AHLs which are positive correlation strongly with the settleability of initial granular sludge were C8-HSL, 3OHC8-HSL and 3OHC12-HSL, especially in R2. Along with the formation of mature granular sludge, only C4-HSL was negative correlation obviously with the stability of granular sludge. Results of 3D-EEM analysis showed that tryptophan and hydrophobic protein-like were major components in the extracellular polymeric substances (EPS) of mature granule, and AHL-producing bacteria such as Xanthomonadaceae were enriched under condition of short SRT. It was speculated that C8-HSL, 3OHC8-HSL and 3OHC12-HSL favor the formation of aerobic granular sludge via the enrichment of functional microorganism and secretion of EPS especially extracellular protein. Conclusions: It is demonstrated that shorter SRT could assemble more AHL-related bacteria and EPS secreting bacteria, which favor the aerobic sludge granulation and then provide a suitable ecological niche for denitrification.
Abstract Title:
The Effect of Starvation on Bacterial Survivability in Sand and Evolution of Biofilms: A Multi-Scale Study

Primary Author Block:
S. Ramezanian, C-L. Kang, N. Abu-Lail; Washington State Univ., Pullman, WA

Abstract Body:
Background: Formation of biofilms in soil offers a sustainable solution for many geotechnical problems such as soil erosion and contamination. However, little is known regarding how and for how long bacterial biofilms are sustained in soil environments with low nutrient availability. Here, the effect of nutrients’ starvation on biofilm growth and evolution of extracellular polymeric substances (EPS) of Pseudomonas putida in sand columns was investigated. Methods: At the macroscale, static biofilms of P. putida were grown under poor (mineral medium) or rich (mineral medium supplemented with glucose (1 g/L)) nutrients’ conditions for 80 days. Temporally, biofilms were assayed for bacterial growth kinetics (colony forming unit (CFU)) and the quantity of proteins and carbohydrates present in their EPS. At the nanoscale, biological force microscopy was used to quantify the adhesion forces acting between the biofilms and a model surface in saline. To prepare the biological probes, P. putida biofilms were grown on tipless cantilevers for 35 days under both poor and rich nutrient’ conditions. Results: Our macroscale results indicated that the CFU count decreased by two fold 21 days after starvation and remained constant after that, while the fed bacterial CFU count reached a maximum at day 20 and stayed constant after that. Carbohydrates’ concentration of EPS decreased gradually during the 80 days of experiment for both fed and starved biofilms with no statistical significant difference between the two. In comparison, the proteins’ concentration of the starved biofilms increased throughout experiment and that for fed biofilms remained constant throughout. The proteins’ concentration of starved biofilms was 4 fold larger than that of the fed biofilms. Based on our results, we hypothesized that cells under starvation likely synthesize proteins to adhere better to sand. To test our hypothesis, nanoscale experiments of adhesion of starved biofilms for 35 days to a model silicon surface were compared to those of biofilms grown under rich nutrient’ conditions. Our results validated our hypothesis and indicated that the adhesion forces measured with starved biofilms were 4 times those measured with fed biofilms. Conclusions: Our results suggest that, for field studies, administering nutrients once every few months will not disturb biofilm function in soil. Moreover, since the bacterial viability was maintained during the 80 days of experiment, it is expected that biofilm will start to form as soon as the carbon source is supplemented.
Mechanical Bactericidal Effects of Nanotopography of Cicada Wings on Escherichia coli

Q. Wan, H. Li, S. Zhang, C. Wang, X. Xu, B. Pan; China Agricultural Univ., Beijing, China

Abstract Body:
Cicada wings are famous for their superhydrophobic and self-cleaning properties. But the wing surfaces cannot limit bacterial adhesion. Electron microscopy showed a periodic array of spherically capped nanopillars on Cryptotympana atrata wings. While investigating the state of Escherichia coli on the wings of the cicada, we found that the nanostructures penetrating the cells, indicating that the wings were actually deadly to the bacteria. The bactericidal effects were confirmed through viability experiments using SYTO 9 and propidium iodide fluorescent dyes. The cicada wings maintained a clean surface through bactericidal action, rather than repelling the bacterial cells. These natural surfaces that inhibit bacterial contamination via their physical structure might have enormous potential for application in the production of biomimetic antibacterial materials. Key words: Cryptotympana atrata; Wings; Nanostructures; Bactericidal; Escherichia coli
In this study, the changes in microbial community profile collected from the foot swabs of cadavers placed at the Anthropological Research Facility (ARF) at the University of Tennessee at Knoxville on October 27, 2016, were examined using Illumina MiSeq NGS of the collective microbial 16S rRNA genes (V4 region) and bioinformatics tools. The plantar surface of each cadaver (n = 3) was sampled in duplicate via sterile cotton swab at Day 1 (A) Day 31 (B) and Day 58 (C). Taxonomic distribution at the highest resolution against the GreenGenes (v13.8) database and alpha diversity were determined using QIIME (v1.8.0). At time A, biological replicates showed Tissierella_Soehngenia to be dominant (up to 48%), along with variable abundances of Lactobacillus, Prevotella, Fusobacterium, Acinetobacter, and members of Clostridiales. Time B also showed these taxa, however the proportional distribution of taxa shifted to show a higher Shannon and Simpson diversity (avg. 7.94 and 0.92, respectively) relative to Time A (avg. 7.12 and 0.90). Time C exhibited a marked increase in Pseudomonas (up to 90%), along with a persistence of Acinetobacter, showing the lowest Shannon and Simpson alpha diversity (avg. 3.68 and 0.68). Beta diversity analyses using Bray-Curtis-based multidimensional scale (MDS) plot analysis showed samples at Time A and B to cluster together (> 35%), separate from Time C (> 25%), and ANOSIM demonstrated significant grouping of microbial community composition in biological replicates (p = 0.001; R = 0.7). Heatmap analysis elaborated the cluster patterns of sample groups, showing Pseudomonas to contribute to the distinct microbial diversity at time C. Oligotyping for fine-scale diversity analysis of 16S rRNA gene sequences revealed a distinct oligotype (Oligotype 1) to comprise the highest proportion of sequences assigned to Pseudomonas at time point C, which was present at very low abundances at A and B (<1%), with a high NCBI BLAST similarity match (100% sequence identity with an e-value of 4E-128) to P. veronii, P. extremaustralis, P. rhodesiae, and P. fluorescens. Overall, these results demonstrate the population dynamics and changes in the microbial taxa occurring on the skin of cadavers in the natural environment at ARF, and show a notable expansion of Pseudomonas (Oligotype 1) at day 58 of decomposition. Such information provides insight into the microbiota likely benefitting from and/or contributing to the human skin decay process post-mortem, and the use of 16S rRNA gene NGS as a potential forensic tool to determine post-mortem interval (PMI).
Ecological Survey of Electrogenic Bacteria Using Microbial Fuel Cells

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Background: A microbial fuel cell is a bioreactor that utilizes microorganisms to convert chemical energy into electrical energy. Mud is commonly used as both a fuel source and an inoculum especially for benthic fuel cells. Bacterial capacity for oxidizing acetate and depositing electrons directly onto the catalytic surface is influenced by environmental conditions, such as mud and soil type, as well as oxygen and carbon availability. Methods: Fuel cells used carbon felt electrodes, wires and a hacker board outfitted with a capacitor and LED light, all from the MudWatt kit (mudwatt.com). Mud was collected from eight locations around San Luis Obispo County: 4 saline, 2 freshwater, 2 dry soils. Anodes were embedded in soil/mud samples and cathodes placed at the top. Wires embedded through both were hooked to the hacker board. Soils were analyzed to determine pH, %C, %N, %H2O, and conductivity. Voltage and power output were monitored daily for 40 days with a multimeter and the MudWatt app respectively, then T/2 (half the time it takes a cell to reach peak output) was determined. Selected muds were autoclaved or salted to examine the effect of lice organisms and salinity on power output. Acetate was injected into selected low power fuel cells to test for C-limitation. Results: Three soils did not produce significant power over a 60 day incubation. Cal Poly Compost soil (12% C) produced the highest output at 640 mV and 146 uW. When injected with acetate, fuel cell output increased by an average of 146 mV in low performing cells. Peak power output was most highly correlated (0.743) to %C. Mud from saline environments had a high electrical conductivity (average value greater than 10 S) which correlated highly with T/2, but not peak power output. Autoclaved muds produced very low power output, which did not increase over time. Autoclaved cells with marine mud produced an average of 200 mV as did freshwater autoclaved samples with added salt. Conclusions: Electrogenic bacteria are not universally present in soils around San Luis Obispo county as some cells produced insignificant power output over 60 days. Soil carbon content in mud-based fuel cells appears to be the main driver of peak power output. High salinity/conductivity soils can facilitate rapid, low-level voltage generation in mud-based fuel cells. Future efforts will focus on using molecular methods to taxonomically identify the electrogenic bacteria enriched on the anodes of our marine and freshwater mud-based fuel cells.
Abstract Title:
Investigation of the Interface of An Electrode-Grown Microbial Biofilm by Electrochemical Surface Plasmon Resonance Spectroscopy

Primary Author Block:
M. D. Yates1, J. P. Golden1, M. Halsted2, L. M. Tender1; 1Naval Res. Lab, Washington, DC, 2The Univ. of Tennessee, Knoxville, Knoxville, TN

Abstract Body:
The interface between biofilms and solid surfaces can be difficult to characterize. For organisms that utilize a solid surface as a metabolic electron donor or acceptor, processes occurring at the interface are particularly important. These biofilms, composed of electroactive organisms, transfer electrons across the cell membrane, through an extracellular matrix, and finally across the interface of an insoluble electron acceptor via a faradaic process. For a faradaic reaction, changing the potential of the electrode induces a change in the relative amounts of oxidized and reduced redox molecules at an electrode surface. Electrochemical surface plasmon resonance spectroscopy (ESPR) monitors faradaic processes optically from the accompanying change in refractive index induced by the change in oxidation state of the redox cofactors at the surface. Here, we demonstrate application of ESPR to investigate the anode-grown Geobacter sulfurreducens biofilm (GSB). The results reveal electrochemical properties of GSB not directly observed by current alone. Most notable is the observation that when the potential is changed from 0.5 V to -0.2 V and back to 0.5 V vs. SHE, an appreciable amount of extracellular electron transport (EET) redox cofactors within 0.5 µm of the electrode surface (ESPRI propagation depth) remain trapped in the reduced form without affecting the magnitude of turnover current, which is proportional to the respiration rate of the biofilm. The results indicate that EET redox cofactors near the electrode can store charge even when the electrode is at an oxidizing potential. These insights will help us further understand the mechanisms used by these organisms to transport charge, which may aid in developing emerging biotechnological applications that utilize electroactive organisms.
Abstract Title:
Electron Flow Rate in Corrosion by Iron-Corrosive Methanogen

Primary Author Block:
S. Wakai; Kobe Univ., Kobe, Japan

Abstract Body:
Background: Corrosion of metal materials by microorganisms is referred to as microbiologically influenced corrosion (MIC). However, electron flow rate of corrosion by an electrically corroding microorganism have not yet been investigated. Iron-corrosive methanogen Methanococcus maripaludis KA1 was isolated from bottom water in a petroleum oil storage tank. It can grow with metallic iron (Fe0) as electron donor and carbon dioxide as electron acceptor. In contrast, non-corroded strain M. maripaludis JJT cannot grow with Fe0. In addition, co-existence of non-corroded sulfate reducing bacterium Desulfovibrio vulgaris with strain KA1 shows enhanced corrosion compared with the corrosion by alone strain KA1. Therefore, we carried out corrosion test using corrosive and non-corrosive strains in order to estimate electron flow rate from Fe0. Methods: M. maripaludis strains KA1 and JJT and D. vulgaris were cultivated with molecular hydrogen as electron donor. Corrosion test was carried out using modified artificial seawater medium containing 30 mM of NaHCO3 and iron foil (10×10×0.1 mm, Fe>99.9%) purged with N2 gas, and each bottle was statically cultivated at 37oC. Methane and hydrogen in the headspace of each vial were quantified on a gas chromatograph. Electron flow rate was calculated from the moles of generated gases. Results: The electron flow rates of corrosive strain KA1 was 56.1 μmol e−/cm2/d during methanogenesis. In contrast, abiotic control without inoculation showed 1.5 μmol e−/cm2/d during H2 generation. The electron flow rates of non-corrosive strain JJT was not calculated because no methane and hydrogen were detected after 42-days cultivation. In addition, we conducted corrosion test using corrosive strain KA1 and D. vulgaris. As a result, the electron flow rate was 131.5 μmol e−/cm2/d during early corrosion phase, and it was approximately 2.3- and 87.7-fold higher than those of KA1 alone and abiotic control, respectively. Conclusions: Under anaerobic condition, Fe0 is slowly corroded in the absence of microorganisms, while iron-corrosive methanogen M. maripaludis KA1 and co-existence with D. vulgaris significantly accelerated electron flow rates from Fe0. Cathodic depolarization theory as corrosion mechanism by hydrogen-consuming microorganisms have been known, while the hydrogen-consuming methanogen M. maripaludis JJT did not accelerate the electron flow rate. Therefore, iron-corrosive methanogen M. maripaludis KA1 would have unique electron flow system from Fe0.
Effect of Microstructured Electrodes on Bacterial Biofilm Signaling for Bioelectrochemical Applications

Primary Author Block:
S. E. Astorga1, L. X. Hu1, E. Marsili2, Y. Z. Huang1; 1Nanyang Technological Univ., Singapore, Singapore, 2Singapore Ctr. for Environmental Life Sci. Engineering (SCELSE), Singapore, Singapore

Abstract Body:
Background: Microbially influenced corrosion, bioenergy production, and biosensors for food, environmental, and clinical applications are interest for bacteria capable of extracellular electron transfer (EET) to insoluble metals and electrodes. The known EET mechanisms rely on direct electron transfer through transmembrane cytochrome complex, microbially produced redox mediators and pili-like conductive appendages termed nanowires. On the other hand, EET process allows rapid and sensitive signaling of bacterial biofilms in potentiostat-controlled electrochemical cells. However, the low coulombic efficiency of a microbial system requires a high surface electrode with high current output. Furthermore, microstructured electrodes allow investigating the EET mechanism in the biofilms.

Methods: Here, a unique bioelectrochemical sensor is presented. Ordered structured array gold electrodes were used as support to grow E. coli biofilms and measure EET rate. Gold electrodes with microstructures of 4 μm and 8 μm diameter gold pillars were fabricated using lithography. The electrodes morphology were characterized using SEM (scanning electron microscopy). The bioelectrochemical characterization included Chronoamperometric (CA), Cyclic Voltammetry (CV), and Electrochemical Impedance Spectroscopy (EIS) which were performed before and after the 24 hr CA. SEM and CLSM (confocal laser scanning microscopy) were employed to observe the bacteria attachment on the electrodes. Results: The arrays offer a larger surface area and promote biofilm formation which increases the EET rate. Biofilm formation is confirmed by SEM and CLSM images. The biocompatible pillar micro-structures provide a continuous pathway for EET from the bacteria (E. coli). In addition, the results show that the modified electrode enhance the current output up to 22% more than the control electrode, hence the electrochemical detection of E. coli biofilms. Such bacterial biomass increment decreases the resistance and increases the capacitance on the electrode surface as proved by the EIS analysis. Conclusion: We successfully improved the biofilm signal detection using a self-fabricated electrode with microstructures. These improved electrodes will potentially benefit high sensitive, rapid and accurate response of biosensors, excellent stability and recyclability.
Abstract Title:
Enhancing the Electrogenic Conditions in A Wastewater Microbial Fuel Cell
Primary Author Block:
J. Romero, T. Roane, J-D. Park;  Univ. of Colorado, Denver, Denver, CO
Abstract Body:
Microbial fuel cells (MFCs) rely on electrogenic bacteria to produce electricity, offering an innovative form of sustainable energy. Despite the increasing number of electrogenic bacteria being identified in domestic wastewater, the microbial conditions needed to produce electricity are still being determined. The objective of this study was to identify MFC conditions to enhance the production of electrical power by wastewater-associated electrogenic bacteria. Conditions examined included (1) the use of iron versus titanium anodes; (2) the use of a 2-aminophenylethyl D-mannose electrophilic coating on an iron anode; and (3) the use of mineral and vitamin components in the electrogenic bacterial growth medium. Within 30 days of incubation under 1000 Ω resistance, the most electrically productive MFC contained an uncoated iron oxide anode and a vitamin and mineral-enriched medium, producing an average of 334 mV. An MFC with a similar medium but a titanium anode produced an average of 24 mV, resulting in a 7-fold difference in power production in comparison to the previous MFC. Fluorescence light microscopy performed on the iron oxide anode fibers of productive reactors showed evidence of a uniformly distributed biofilm, while biofilms associated with less productive reactors were found to be randomly distributed or nonexistent. The diversity of the anode biofilm of several MFCs was confirmed via Illumina high throughput 16S rDNA sequencing. The solutions surrounding the anodes, thought to contain important members of an MFC’s overall electrogenic community, were equally diverse with slight differences in abundances of the bacterial groups. Finally, the bacterial communities were found to change over the four months from reactor set-up to peak performance. Both anode and solution communities of the productive reactors were typically dominated by members of the Enterobacteriaceae family (>70% of the identified species). Over time, both communities became dominated by members of Pseudomonadaceae and Rhodocyclaceae, known electrogenic families (14-30% and 18-21%, respectively). Less productive reactors did not develop into similarly dominated communities, instead being dominated by groups such as Lactobacillaceae and Comamonadaceae. Results to date show how reactor modifications can influence the activity of electrogenic bacteria. Continuing work will identify influencing conditions towards the goal of MFC optimization for enhanced power production to ensure the successful development of this technology.
Abstract Title:
Anodic Electro-Fermentation of Acetoin in A Metabolically Engineered E. coli Strain

Primary Author Block:
S. Beblawy, A. Förster, J. Gescher; Karlsruhe Inst. of Technology, Karlsruhe, Germany

Abstract Body:
Background: Electro-fermentation is a new strategy in anaerobic biotechnology. It can be used for reactions in which the average oxidation state of the end product is higher than the substrate. Conventionally, these reactions are catalyzed under oxic process conditions accompanied by higher biomass formation and high energy input into the system. The goal of this study was to establish an electrode-assisted fermentation process in E. coli for the strictly anoxic production of acetoin. The surplus of electrons released in the production process is transferred to an electrode as anoxic and non-deplatable electron acceptor. Results: The central metabolism was steered towards the production of pyruvate from glucose by deletion of genes encoding for enzymes of central reactions of the anaerobic carbon metabolism (ΔfrdA-D ΔadhE ΔldhA Δpta-ack). Thereafter, the heterologous expression of the acetolactate synthase (alsS) and the acetolactate decarboxylase (alsD) facilitated the formation of acetoin. The fermentation with nitrate as electron acceptor lead to an anaerobic acetoin production with a yield of up to 90% of the theoretical maximum. The expression c-type cytochromes from Shewanella oneidensis and the addition of the soluble redox-shuttle methylene blue allowed for the replacement of nitrate as electron acceptor by a carbon electrode. The interaction with the non-deplatable electron acceptor lead to an acetoin formation with a yield of 79% of the theoretical maximum. Conclusion: Here, we show for the first time a process in which the commonly used chassis strain E. coli was tailored for an electrode-assisted fermentation approach branching off from the central metabolite pyruvate. At this early stage, we see promising results regarding carbon and electron recovery. However, the productivity of this system is rather low. Further strain development and improved fermentation conditions will be used to increase the anaerobic metabolic turnover rate, to an extent that allows for an applicable biofilm-based production process.
Abstract:

Background: When a Shewanella sp. utilizes lactate, the metabolism diverged from acetyl-CoA can either enter the tricarboxylic acid (TCA) cycle, or can be transformed into acetate to produce adenosine triphosphate (ATP). In our recent study, we found that on a Shewanella anode (poised at +0.63 V vs. SHE), all the electrons derived from the TCA cycle were used for cell growth, but not for current generation. To clarify the interaction between central metabolism and phosphorylation during extracellular-electron transfer (EET) of Shewanella sp., it is important to test the current generation using different substrates. Methods: The strain S. decolorationis NTOU1 was isolated from a water cooling pipeline. The electrochemical cells were connected to a potentiostat with poised working electrode. The organic acids were detected using high-performance liquid chromatography. ATP quantification was performed using ATP/ADP ratio Assay Kit. Results: When using the 35 mM acetate as a substrate for S. decolorationis NTOU1, only 2 mM acetate can be digested and only 5% of electrons can be recovered for electric-current generation in 72 h. While knowing that the mediator addition can boost EET, different mediators (i.e., riboflavin and ferricyanide) were externally spiked in the electrochemical cell and the current changes were recorded. According to the results shown in the image 1, the riboflavin spiking dose not significantly boost the current generation, but 100 μM ferricyanide spiking could enhance the current from 150 to 550 μA. Conclusions: 1. The riboflavin is a proton-associated redox compound (i.e., ferricyanide is not) that might scavenge all to proton in the periplasm and thus lead to less ATP production and unfavorable acetate degradation. 2. The ferricyanide possesses a higher formal potential (+0.36 V vs. SHE) than riboflavin does (-0.3 V vs. SHE), which can better favor the electron transfers through the outer-membrane cytochrome on a Shewanella cell.
Abstract Title:
In Silico Characterization of A Novel Geopilin from Ferruginous Lake Sediments

Primary Author Block:
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Abstract Body:
Molecular mechanisms for microbial Fe(III) reduction are important in the fields of biogeochemistry and biotechnology. The order Desulfuromonadales within the Delta proteobacteria contains many Fe(III) reducers, including Geobacter spp. These organisms use a conduit of multiheme cytochromes to shuttle electrons to the outer membrane, whereupon some members pass them to modified type IV pili, or “geopili” to directly contact and reduce Fe(III) particles. Recently, pilins of diverse size and structure were proven to be conductive provided they maintain a high density of aromatic amino acids in the pilus structure. In this study a novel pilin gene was characterized from an incubation of anoxic, Fe(III)-rich lake sediments. Sediments from Lake Matano Indonesia, a stratified lake with anoxic sediments, were incubated with ferrihydrite and CH4 headspace to mimic situ conditions. After 15 days, DNA was extracted and 16S rRNA amplicon and metagenomic sequencing were performed. The metagenome was probed for Fe(III)-reducing machinery. An unidentified member of Desulfuromonadales dominated the incubations based on 16S sequencing and a near complete metagenome-assembled genome corresponding to this organism was recovered. Phylogenetic analysis of the rpoB gene suggests that this genome may represent a new genus. The genome contains homologs to 37 multiheme cytochromes localized to both the inner and outer membranes as well as the periplasm. The geopilin encoded by this genome was significantly longer (198 aa) than those from Geobacter spp. However, it boasts a high density of aromatic residues throughout its length. We have identified an organism from lake sediment with the potential to reduce Fe(III) using a structurally distinct geopilus, making this organism of particular interest to biotechnology as these pili allow for better growth and current production in microbial fuel cells. Further work is needed to better characterize this organism and its pilus.
Abstract Title:
Diet As A Possible Path for Syntrophic Oxidation of Fatty Acids
Primary Author Block:
Y. Lu; Peking Univ., Beijing, China
Abstract Body:
Background: Fermentation in anoxic environments produces intermediate products including various short-chain fatty acids and alcohols. A sophisticated interspecies electron-transfer (IET) network is formed for methanogenesis with H2 and formate as common known electron-carriers. The discovery of direct interspecies electron transfer (DIET) in Geobacter however has put forward a challenge to the classical theory. We provide multiple evidences to illustrate that DIET is possible in canonical syntrophy. Methods: 1) Environmental samples from paddy field soil, natural wetland and lake sediment were used for enrichment cultivation in the presence of butyrate; 2) Physiology tests were conducted to determine the effects of conductive materials including magnetite and carbon nanomaterials on different enrichments and a defined coculture comprising canonical syntroph Syntrophomonas wolfei and Methanococcus Maripaludis; 3) Molecular analysis was conducted to determine microbial compositions in enrichments; 4) FISH and scanning electron microscope (SEM) observation was carried out to analyze the spatial arrangement of syntrophic organisms. Results: In all enrichment incubations, a substantial stimulatory effect was consistently detected on butyrate oxidation in the presence of magnetite nanoparticles (nanoFe3O4) and carbon nanomaterials. This effect remains when different conductive nanomaterials are interchanged in enrichments. This effect however disappears if nanoFe3O4 is coated with silica SiO2 that insulates the mineral conductivity or if carbon nanotubes are replaced by kaolinite, a clay mineral without electric conductivity. Test on pure culture M. maripaludis reveals zero to negative effect by carbon nanomaterials. Molecular analysis reveals that the presence of Geobacter is not essential for the positive effect. FISH and SEM images reveals the formation of cell-nanomaterial mixture aggregates. But otherwise, bacteria and methanogen cells appear more separated in the presence of conductive nanomaterials and hence increasing the diffusion barrier for canonical interspecies H2/formate transfer. Conclusions: Collectively, our data suggest that the presence of conductive nanomaterials likely induces DIET in syntrophic butyrate oxidation. Albeit the lacking of e-pili-like structure and outer-membrane cytochrome, a provision of externally conductive materials may set a substitution opportunity for syntrophy organisms. More studies are needed to elucidate the probable mechanisms.
Simultaneous Bioremediation and Energy Recovery from Abattoir Wastewater Using Copper Anode Microbial Fuel Cell

D. O. Fasheun, E. C. Egwim; Federal Univ. of Technology Minna, Niger, Nigeria

Background: The anode material of a microbial fuel cell (MFC) is very important to its function. Carbon in its different forms, is the most commonly used anode material for electrochemical systems because of its biocompatibility but it suffers a major setback as it has a lower electrical conductivity and higher resistivity than that of metals. Copper is a good conductor but its use in microbial fuel cell (MFC) is limited due to its antimicrobial properties. Abattoir wastewater contains large volumes of organic matter coming from the cow blood, rumen contents and wash water. In many cases, these residual effluents are discharged directly into water bodies thereby putting these ecosystems at risk. In this study, the potential of bioremediation and energy recovery from abattoir wastewater using copper anode MFC was therefore determined.

Methods: Four dual chamber (separated by a rubber latex proton exchange membrane; 3cm x 0.12mm) MFCs were constructed with eight transparent plastic containers (1.2 litre volume each). The anode was Copper coil (28cm x 1.5mm) while the cathode was Aluminium mesh (16cm x 11cm x 1.5mm). Abattoir wastewater served as both the inoculum and organic substrate source for the MFC. A digital multimeter was connected to the MFCs to monitor the open circuit voltage and the voltage across 100ohms, 220ohms, 470ohms and 1000ohms resistors for 8 days at 24hour interval. The corresponding power and power densities were calculated. Bacterial community analysis of the anodic biofilm, raw and treated abattoir wastewater was carried out to check for microbial dynamics. Results: The MFC produced an open circuit voltage of 0.895±0.009V. When connected to different resistors, the MFC with the 1000Ω resistor produced the most stable power with a peak voltage and power density of 35mV and 925.8mW/m2 respectively on day 6. The bacterial community analysis of the abattoir wastewater revealed the presence of Bacillus Subtilis, Bacillus megaterium, Staphylococcus aureus, Klebsiella pneumonea, Escherichia coli, and Micrococcus luteus, while only Bacillus Subtilis, and Bacillus megaterium were present in the biofilm formed on the copper anode. After 8 days of operation, the abattoir wastewater became odourless, clearer and electrodes became visible. Conclusions: This paper demonstrates that despite the antimicrobial properties of copper, exoelectrogens such as Bacillus Subtilis and Bacillus megaterium can form biofilm on the copper anode and facilitate the simultaneous bioremediation and energy recovery from abattoir wastewater.
Abstract Title:
A Mechanism for Metal Reduction in Aeromonas Hydrophila

Primary Author Block:
B. E. Conley, J. A. Gralnick, D. R. Bond; Univ. of Microbiology, St. Paul, MN

Abstract Body:
Many microorganisms are capable of using a variety of compounds as terminal electron acceptors. One strategy used by dissimilatory metal reducing bacteria involves donating electrons to insoluble metals such as Fe(III) and Mn(IV) oxide minerals or poised electrodes. Electron transfer to these extracellular substrates requires unique pathways to move electrons from inside the cell to the extracellular terminal electron acceptor. There are two model microorganisms for which the mechanism of metal reduction is relatively well understood: the δ-Proteobacterium, Geobacter sulfurreducens, and the γ-Proteobacterium, Shewanella oneidensis. However, there are many diverse microorganisms that have been reported to reduce metals, yet experimental insight into their mechanisms of extracellular electron transfer is lacking. Based on previous studies and genomic comparisons, we hypothesized the γ-Proteobacterium Aeromonas hydrophila uses a unique mechanism for extracellular electron transfer. Genes potentially involved in metal reduction were deleted from wild-type A. hydrophila and were heterologously expressed in S. oneidensis mutant backgrounds. Extracellular electron transfer was assayed by measuring Fe(III) and Mn(IV) reduction in A. hydrophila and S. oneidensis mutants. A novel inner membrane system which resembles formate dependent nitrite reduction components (NrfBCD) and a unique periplasmic di-heme cytochrome were identified as required for extracellular electron transfer in A. hydrophila. Additionally, Shewanella-like outer membrane proteins (MtrCAB) are also required for metal respiration. The entire A. hydrophila metal reduction pathway functionally complemented an S. oneidensis mutant unable to reduce metals. Identifying novel genetic signatures encoding metal reducing components in a diverse range of microorganisms will help determine the distribution of this metabolism in the environment. Understanding the range of dissimilatory metal reducing bacteria will help elucidate the contribution of microbial metabolism to environmental metal cycling, and potentially provide more efficient systems for industrial applications.
Abstract Title:
Bacteriological Safety of Sachet Drinking Water Sold in Benin City, Nigeria
Primary Author Block:
S. O. Akintayo; Univ. of Ibadan, Ibadan, Nigeria
Abstract Body:
Access to safe drinking water remains a major challenge in Nigeria, and where available, the quality of the water is often in doubt. An alternative to the inadequate clean drinking water is being found in treated drinking water packaged in electrically heated sealed nylon and commonly referred to as “sachet water”. “Sachet water” is a common thing in Nigeria as the selling price is within the reach of members of the low socio-economic class and the setting up of a production unit does not require huge capital input. The bacteriological quality of selected 80 “sachet water” stored at room temperature over a period of 56 days was determined to evaluate the safety of the sachet drinking water. Test for the detection of coliform bacteria was performed and the result showed no coliform bacteria that indicates the absence of fecal contamination throughout 56 days. Heterotrophic plate count (HPC) was done at an interval 14 days, and the samples showed HPC between 0 cfu/mL and 64 cfu/mL. The highest count was observed on day 1. The count decreased between day 1 and 28, while no growths were observed between day 42 and 56. The decrease in HPC suggested the presence of residual disinfectant in the water. The organisms isolated were identified as Staphylococcus epidermis and S. aureus. The presence of these microorganisms in sachet water is indicative for contamination during processing and handling.
Abstract Title:
Prevalence of Tetracycline Resistance Genes among Multi-Drug Resistant Bacteria from Selected Water Distribution Sys. in Southwestern Nigeria

Abstract Body:
Background: Antibiotic resistance genes [ARGs] in aquatic systems have drawn increasing attention they could be transferred horizontally to pathogenic bacteria. Water treatment plants (WTPs) are intended to provide quality and widely available water to the local populace they serve. However, WTPs in developing countries may not be dependable for clean water and they could serve as points of dissemination for antibiotic resistant bacteria. Only a few studies have investigated the occurrence of ARGs among these bacteria including tetracycline resistance genes in water distribution systems in Nigeria. Methodology: Multi-drug resistant (MDR) bacteria, including resistance to tetracycline, were isolated from treated and untreated water distribution systems in southwest Nigeria. MDR bacteria were resistant to >3 classes of antibiotics based on break-point assays. Isolates were characterized using partial 16S rDNA sequencing and PCR assays for six tetracycline-resistance genes. Plasmid conjugation was evaluated using E. coli strain DH5α as the recipient strain. Results: Out of the 105 bacteria, 85 (81 %) and 20 (19 %) were Gram- negative or Gram- positive, respectively. Twenty-nine isolates carried at least one of the targeted tetracycline resistance genes including strains of Aeromonas, Alcaligenes, Bacillus, Klebsiella, Leucobacter, Morganella, Proteus and a sequence matching a previously uncultured bacteria. Tet(A) was the most prevalent (16/29) followed by tet(E) (4/29) and tet30 (2/29). Tet(O) was not detected in any of the isolates. Tet(A) was mostly found with Alcaligenes strains (9/10) and a combination of more than one resistance gene was observed only amongst Alcaligenes strains [tet(A) + tet30 (2/10), tet(A) + tet(E) (3/10), tet(E) + tet(M) (1/10), tet(E) + tet30 (1/10)]. Tet(A) was transferred by conjugation for five Alcaligenes and two E. coli isolates. Conclusions: This study found a high prevalence of plasmid-encoded tet(A) among Alcaligenes isolates, raising the possibility that this strain could shuttle resistance plasmids to pathogenic bacteria.
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Session Primary Track: Applied and Environmental Science
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Abstract Title:
Bacteriological Analyses of Commercial Sachet Water in A Tertiary Inst. Campus in North West Nigeria
Primary Author Block:
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Abstract Body:
A study was conducted to analyze the quality of some selected brands of sachet water used for drinking which are sold on the campus of Federal College of Education, Zaria, Kaduna state, Nigeria. Fifteen (15) popular brands were identified and selected based on patronage by consumers and distributors. Triplicates of each brand were analyzed with a total of 45 samples. Analysis was performed using culture and biochemical on coliforms. Each water sample was serial diluted and inoculated in three tubes of MacConkey broth (10ml, 1ml, 0.1ml). All tubes were incubated at 370C for 24hours. The fifteen different brands were labeled A-O for ethical reasons. The result of the Presumptive test to show the total number of Coliforms present in the sample using the most probable number (MPN) technique (/ml) showed that sample A and O had the highest number with 18ml, while sample I, L and N had 16ml, D had 9ml, B had 5ml and sample G had 2ml. No Coliform was found in C, E, F, H, J, K and M. The Confirmation test showed that Sample A, I, L and N were positive when the samples were inoculated on EMB (eosin methylene blue) agar plates. The biochemical reactions to indicate completion of the tests from sample indicated the presence of Escherichia coli (23%). Other identified microorganisms from the positive samples include Kleibsella spp (17%), Proteus spp (10%) Pseudomonas spp (25%) and Chromobacterium violaceum (3%). It is imperative to carry out a periodical bacteriological assessment of commercially available packaged sachet water to ascertain its quality before being sold to the public.
Abstract Title:
Health Risk of Drinking Water Sources in Some Agrarian Rural Communities in Sw Nigeria
Primary Author Block:
K. Eniola1, S. Awe2, T. M. Kayode-Isola1, P. O. Oluwasola1, S. Abraham1, B. Obajimi1, O. Abioye1;
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Abstract Body:
Water of acceptable quality is important to improved standard of living. Portable water is particularly important in rural communities where access to health care is poor. Goal 6 of the sustainable development goals (SDGs) is to ensure availability and sustainable management of water and sanitation for all. This study reports probable health risk of major drinking water sources in four (4) agrarian rural communities: Ipetu-Ijesa, Ikeji Arakeji, Owena-Ijesa and Ita reserve in Oriade LGA, Osun state, Southwest Nigeria. Twenty four community water sources (nineteen (19) wells, four (4) boreholes and one (1) stream) were identified, and examined using defined substrate technology (Colilert and Petrifilm tests). The stream, wells (19) and two boreholes were found to contain coliform (ONPG positive), further examination showed the stream and seventeen (17) wells contained E. coli (MUG positive). The stream and nine (9) wells were also found to contain more than 10 E.coli/ml, eight (8) wells contained between 1 and 10 E.coli/ml, while two (2) wells and the four boreholes had counts of zero (0). The results suggest high to very high risk level of disease occurring by drinking water from most water sources available to the communities. Only six (6) of the water sources were fit for consumption (low risk level of disease). This study reveals the need for interventions if the SDG goal 6 is to be realized in these areas because unsafe drinking water sources are still being used in the Communities. Safe drinking water sources should be provided to guarantee health of Communities and improve productivity.
Abstract Title:
Virulence and Resistance Profile of Vibrio Species from Self-Supplied Hand-Dug Well Sys. in Two Suburban Communities of Lagos Nigeria

Primary Author Block:
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Abstract Body:
Background: Well water meant for consumption must meet the quality guidelines for drinking water to reduce possible health-risk associated with chemical hazard and pathogenic microorganisms such as Vibrio species. Methods: Water in thirty hand-dug wells sited close to septic tanks in Ojo and Badagry area of Lagos, Nigeria were examined for Vibrio species using membrane filtration techniques on TCBS agar after enrichment in Alkaline Peptone Water. The Vibrio isolates were evaluated for multidrug resistance using disc diffusion method and their plasmid profile assayed. Results: Vibrio species were found in all the wells, a total of one hundred and thirty one (131) strains belonging to five (5) species of Vibrio were obtained. These are: V. furnissii (58), V. vulnificus (24), V. harveyi (15), V. anguillarum (12), V. parahaemolyticus (6), V. mimicus (12) V. cholera (4). V. furnissii strains were the most encountered, they were found in twenty three (23) of the wells, V. anguillarum and V. parahaemolyticus were the least encountered strains; they were found in only one well each. V. cholera strains were found in four (4) of the wells. Seventy-four percent of the Vibrio strains showed multiple drug resistance to Penicillin, Cephalosporin, Chloramphenicol and Cotrimoxazole classes of antibiotics. Fifty-three (41%) possessed plasmid DNA with molecular weight varying between 690 kbp and 974 kbp. A strain of Vibrio cholerae isolated from a well in Ilogbo (Ojo) possessed the virulence gene ctx with molecular weight 161bp. Conclusions: Contamination of well water by Vibrio species is clearly evident, this and detection of multi drug resistance in Vibrio furnissii can be linked to the generally unhygienic state of the catchments of the wells. Hence, point of use interventions such as use of hypochlorite or boiling would be required to make the water safe for drinking.
Comparative Analyses of the Microbiological and Physicochemical Parameters of Lisaluwa River and Three Close Well Water Sources in Ondo City

Background: Water is unarguably one of the most important resources to man. It is necessary for the continual propagation of life. Since, government-provided pipe-borne water are usually lacking, individual household digs their wells for water supply. Although these wells are assumed to be clean, healthy and free of pathogenic microorganisms, there are many ways through which they can become contaminated. For instance, wrong location, poor construction, improper use of wells together with the choice of depth can all contribute to the deterioration of the well water quality (Egbulem, 2003).

Methods: Four water samples from four different sources including Lisaluwa River, Ondo and three well water sources along the course of the river were analyzed for their physicochemical and microbiological qualities. Results: Results of the physicochemical qualities revealed that the pH, turbidity, dissolved solids, dissolved oxygen, temperature and anions concentrations like the phosphate, sulphate and nitrate assessed had low values below the WHO standards. The total bacterial count ranged from 0 to 120 CFU/ml. The bacterial isolates included genera Enterobacter sp, Proteus sp, Escherichia sp, Staphylococcus sp, Pseudomonas sp, Bacillus sp, Shigella sp, Klebsiella sp, Serratia sp, Salmonella sp and Flavobacterium sp while isolated fungal genera included Penicillium sp, Curvularia sp, Aspergillus sp, Fusarium sp, and Nigrospora sp. Conclusions: It was concluded that the high bacterial count and presence of coliforms in the river and well water samples could result in the outbreaks of water-borne illnesses in the community. Therefore, constant monitoring of Lisaluwa river by appropriate regulatory bodies, and purification of well water before consumption are recommended.
Studies on Parasitic Contamination of Soil and Local Drinking Water Source in Doma Local Government Area of Nasarawa State, Nigeria

Primary Author Block:
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Abstract Body:
Water is a natural resource that is essential to all living things on earth, but if contaminated can pose a lot of risk to human health when consumed. This study evaluates the parasitic contamination of drinking water sources and surrounding soil in Doma Local Government Area of Nasarawa State, Nigeria. A total of 48 water and soil samples around water sources were collected from different selected sources (wells, streams and boreholes) between the months of March to July 2017. The water samples were analyzed using the Calcium carbonate (CaCO3) floatation method while a modified Baermann technique was used to examine the soil samples microscopically for the presences of parasites. A total of 32 water samples were found with parasites. These include 2 species belonging to the protozoan group (Entamoeba histolytica, and Giardia lamblia) 3 from the nematode group (Trichuris trichiuria, Ascaris lumbricoides and Hookworm), and 1 trematode group (Fasciola hepatica). The nematode group had the highest contamination rate 19 (59.38%) followed by the protozoan group 12 (37.50%) while the trematode was least with 1 (3.13%). The most contamination was in stream water sources for late dry and early wet season with 69.23% and 94.74% respectively. The wells had 30.77% and 5.26% in late dry and early wet season respectively. The boreholes had zero contamination for both seasons. Prevalence of parasites in relation to sources of water showed a high significant differences ($\chi^2 = 49.741$, df = 2, $P = 0.0000001$), while there was no significant difference in relation to late dry and early rainy seasons ($\chi^2 = 2.3438$, df = 1, $P = 0.1258$). Geohelminths was highest around the borehole area 15 (35.71%) followed by the well area 14 (33.33%) and was least encountered in the stream area 13 (30.95%). However, there was no significant difference ($\chi^2 = 0.94915$, df = 2, $P = 0.6221$) in the prevalence of geohelminths in relation to sources of water area. Result indicated high rate of parasitic contamination of soil and drinking water sources in the study area. There is need for advocacy and enlightenment on the importance of proper drinking water treatment.
Abstract Title:
Presence of Enteric Viruses and Protozoa in Different Sources of Water in the Kathmandu Valley, Nepal
Primary Author Block:
S. Tandukar1, B. Malla1, R. Ghaju Shrestha1, D. Bhandari2, J. B. Shercha1, E. Haramoto1; 1Univ. of Yamanashi, Kofu, Japan, 2Inst. of Med., Kathmandu, Nepal
Abstract Body:
Waterborne pathogens are a major public health concern in developing countries. This study was conducted to evaluate the microbiological quality of different sources of water used for drinking and other domestic purposes in the Kathmandu Valley, Nepal. A total of 115 water samples were collected from 6 different water sources (i.e., deep tube wells (n = 20), shallow dug wells (n = 50), shallow tube wells (n = 20), rivers (n = 8), springs (n = 10), and stone spouts (n = 7)) in wet and dry seasons of 2016. Human adenoviruses, JC and BK polyomaviruses, enteroviruses, and noroviruses of genogroup I were detected by (reverse transcription (RT)-) quantitative PCR (qPCR) in 11 (10%), 16 (14%), 6 (5%), 3 (3%), and 17 (15%) of the 115 samples, respectively. As potential index viruses, pepper mild mottle virus (PMMoV) and tobacco mosaic virus (TMV) were analyzed by RT-qPCR, resulting in positive ratios of 60% (69/115) and 71% (82/115), respectively. Cryptosporidium was not detected in any of the tested samples, whereas Giardia was found in 5 (4%) samples. Of the 115 samples tested, at least one of the pathogen types was detected in 35 (30%) samples. Based on water type, the pathogens were detected with the highest positive ratio (100%, 8/8) in river water samples, followed by shallow dug wells (36%, 18/50), stone spouts (29%, 2/7), and shallow tube wells (20%, 4/20). There was no significant difference in the positive ratios of pathogens in groundwater between the wet (27%, 15/56) and dry seasons (25%, 13/51) (χ2-test, P > 0.05). Escherichia coli, PMMoV, and TMV were judged insufficient to indicate the presence of waterborne pathogens in groundwater sources.
Spatial and Temporal Variability of Bacterial and Archaeal Communities in Rapid Sand Filters

Primary Author Block:
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Abstract Body:
Rapid sand filters (RSFs) not only play an important role in improving the quality of water through filtration at drinking water treatment plants but also influence the structure and composition of downstream microbial communities at the consumers’ taps. Little is, however, known about the temporal variability and spatial heterogeneity of these communities associated with RSFs. This is particularly relevant, as changes in RSF prokaryotic community may have an effect on microbial-mediated drinking water treatment and post-treatment water quality. To address this knowledge gap, we conducted a 4-month survey in a large-scale drinking water treatment plant to assess the extent of spatial and temporal variation in the microbial communities located: (i) along the surface and depth of the RSF bed, (ii) across parallel RSFs within a filter gallery, and (iii) across filter galleries. Specifically, we collected filter bed media from nine randomly selected RSFs harbored within the filter galleries of three purification stations, and analyzed 188 samples through 16S rRNA gene sequence profiling. Our data revealed that the characterized RSFs comprise of compositionally diverse prokaryotic communities, encompassing 5708 OTUs that were distributed across 61 phyla. Despite this high diversity, we have identified 44 operational taxonomic units (OUTs) that accounted for 95.00% of the total sequences, which were mainly represented by Proteobacteria (mean relative abundance, MRA = 89.25%), Bacteroidetes (MRA = 10.14%) and Acinetobacter (MRA = 0.40%). Within Proteobacteria, Aeromonas and Acinetobacter were the two most dominant genera and accounted for 33.11% and 31.08% of the total sequences, respectively. Furthermore, spatial (local, within filter; intra, within filter gallery and inter, between filter galleries) and temporal variation in the membership and structure of the prokaryotic community was primarily attributable to the medium of rare abundant OTUs and to changes in the relative abundance of the dominant OTUs. These spatial and temporal data suggest ecological succession patterns within RSFs and highlights the importance of assessing the impact of these succession patterns on overall filter performance.
Persistence of Salmonella Typhimurium in Well Waters from A Rural Area Located in Changchun, China

J. Li, M. Ding, Z. Han, J. Ma; Jilin Univ., Changchun, China

Persistence of Salmonella Typhimurium ATCC 14028 in 15 well waters from a rural area in Changchun, China was investigated. Results illustrated that in different well water samples, S. Typhimurium showed distinct survival profiles, including survival time (ttd), first log reduction time (δ), and shape parameters (p) of survival curve. Overall, the survival times in all samples ranged from 15 to 80 days when inoculation size was around 10^4 CFU/ml. Principal component analysis revealed that ttds were related with well water physicochemical properties, further multiple step-wise regression analysis showed that ttds of S. Typhimurium in well waters could be best predicted by NH4+-N and pH of well waters. Canonical correspondence analysis and variation partitioning analysis revealed that NH4+-N and pH could explain 27.60% of overall variation of the survival behavior. Our data showed that S. Typhimurium could persist more than 2.5 months in well waters, thus pose a significant health risk to the local communities, actions would be needed to minimize the potential infection outbreaks caused by this pathogen.
Safety of Drinking Water in Nsukka, Southeast Nigeria - An Assessment of Water Quality, Antibiotic Resistance and Risk of Diarrhoea

Primary Author Block:
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Abstract Body:
Background: Access to safe drinking water is essential to health but potable water is in short supply. Nsukka, in southeast Nigeria, is a water-scarce town that depends largely on roof-harvested rain water (RHRW) and tanker-supplied borehole water (TSBW) for drinking and domestic purposes. The aim of this study was to evaluate the bacteriological quality of tanker-supplied borehole water and roof-harvested rain water and quantify the potential public health risks associated with their use. Methods: Over a 5-month period (Feb - Jun 2015), water samples were collected following Standard methods. Faecal indicator bacteria (FIB), including total coliform (TC), faecal coliform (FC) and Escherichia coli (EC), were enumerated by the membrane filtration technique, using Chromocult coliform agar (CCA). The confirmation of E. coli was achieved by polymerase chain reaction (PCR) detection of the target beta-glucuronidase (uidA) gene. Antimicrobial susceptibility of all isolates was determined using the standard Kirby-Bauer disk diffusion method, and analysed according to Clinical and Laboratory Standard Institute guidelines. Quantitative microbial risk assessment (QMRA) was done using the β-Poisson model. Results: A total of 250 water samples (150 TSBW and 100 RHRW) were analysed. The FIB counts were high (ranged as follows: 0 – 4.4×10^3, 0 – 8.1×10^3 and 1.1×10^3 – 9.6×10^3 cfu/100 ml for EC, FC and TC respectively) and mostly exceeded the recommended maximum values suggested by national and international guidelines for safe drinking and domestic applications. Of a total of 87 isolates confirmed as E. coli, 58.6% were multidrug resistant. Significantly (P<0.05) higher concentrations of TC, FC and EC were observed for TSBW and water stored in metal tanks than for RHRW and water stored in plastic tanks. Daily risks of enteropathogenic E. coli (EPEC) infection in individuals exposed to both TSBW and RHRW via drinking and bathing ranged from 1.2×10^-5 to 7.1×10^-4. The yearly risks of infection from waters were unacceptably high (range: 4.3×10^-3 to 2.3×10^-1), exceeding the acceptable risk of 0.01 % (10^-4 infection/person/year), and the guideline value used as by several nations for drinking water. Conclusions: These results provide valuable information regarding the quality of drinkable water. These results show that the bacteriological qualities of drinkable water types in Nsukka are not safe for use without treatment. These waters represent significant public health hazards. This study highlights the need for treatment of these waters before use.
Abstract Title:
Microbiota of Surface Water Sourced from Dairy Farms in South Western Ontario

Primary Author Block:
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Abstract Body:
Background: Surface waters used in dairy operations play an important role in milk safety and the dairy cow’s health. The present study characterized the diversity of microbiota of untreated surface waters used in dairy farms. Method: Fifteen dairy farms, designated as A-O, were selected for bacteriological analysis. Water samples were collected biweekly from December 2015-March 2016 and then monthly from April 2016 to October 2016. Total bacteriological plate counts were performed by the standard membrane filtration method using Sorbitol MacConkey Agar (SMAC) for colony count, Chromocult Coliform Agar (CCA) and CCA + 4 μg/mL ceftiofur to screen resistant E. coli. For 16S rRNA gene sequencing and analysis, total DNA was extracted from surface waters filtered membranes of 14 farms sampled from August to December 2016. Results: Significant differences in colony forming unit per 100 ml (CFU/100ml) were detected between each dairy farm over the sampling period. The CFU counts on SMAC varied from 2.48 to 4.56 log10 CFU/100ml. E. coli was frequently isolated from water of farms B, F and I while Klebsiella spp. were more prevalent in the water from farms F, G H, and I in comparison to the other farms. The total taxonomic composition across all farms at the phylum level was dominated by Proteobacteria (34%), Cyanobacteria (21.8%), Bacteroides (17.6%), Actinobacteria (17.3%) and Verrucomicrobia (5.5%), whereas Nitrospirae, Spirochaetes, Thermi, Acidobacteria, Firmicutes, Gemmatimonadetes, Armatimonadetes, Chlorobi, Planctomycetes and Chloroflexi were in lower abundance (0.1 - 1%); however there was substantial variation between farms. Principal co-ordinate analysis (PCoA) of unweighted UniFrac distance matrices revealed clustering of the different collection time-points within the majority of farms, as well as a broader clustering of different time-points. The results indicate the presence of farm-specific surface water microbiota that underwent similar seasonal compositional changes. No significant differences in alpha-diversity (PD whole tree; p<0.05) were observed between any of the farms. Conclusion: Data showed diversity of bacterial communities in surface waters, both quantitatively and qualitatively on dairy farms in the Southwest Ontario that may help in developing efficient water treatment systems to preserve food safety and animal health.
Phylogenetic Diversity of Escherichia coli Isolated from Mid-Atlantic Irrigation Water Sources: A Conserve Project

Primary Author Block:
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Escherichia coli is often used as an indicator for fecal contamination or presence of pathogens in recreational and agricultural water, respectively. Irrigation water can be a source of E. coli contamination for fruits and vegetables intended for human consumption leading to human illness. The phylogenetic diversity of E. coli in irrigation water has not been extensively evaluated. E. coli isolates can be grouped into four major (A, B1, B2, D) different phylogenetic groups, with B1 harboring shiga-toxigenic E. coli (STEC) and groups B2 and D harboring extra-intestinal pathogenic (ExPEC) that can cause human illness. The objective of this study was to determine the diversity of E. coli in recycled and surface irrigation water sources in the Mid-Atlantic U.S. and to determine the prevalence of beta-lactamase (blaCTX-M) resistance in these E. coli isolates. Eighty E. coli isolates were recovered from 7 different irrigation water sources: 3 non-tidal fresh water (NF) sites, 3 recycled water (RW), and 1 fresh tidal (FT) water over 12 different sampling dates using EPA method 1604 on mI agar. Colonies from each sampling event were randomly selected, DNA, extracted from each colony, and the presence of the uidA gene was confirmed by PCR. Phylogenetic classification was determined using a PCR typing scheme, and the presence of blaCTX-M was assayed by universal CTX-MU primers. Overall, 54% (n=43), 24% (n=19), 12% (n=10), and 10% (n=8) of isolates belonged to groups B1, D, B2, and A, respectively. Only one E. coli isolate (group D, RW site) was positive for blaCTX-M. For E. coli isolates obtained from RW sources (n=31), 67%, 13%, 10%, and 10% were classified as B1, B2, D, and A, respectively. For isolates obtained from NF (n=29) sources, 56%, 24%, 17% and 3% were classified as B1, D, B2, and A, respectively. These results show that irrigation water sources possess a phylogenetically diverse reservoir of E. coli with potential to cause intestinal and extra-intestinal disease. Further examinations of blaCTX-M plasmids from additional E. coli isolates in this study will be conducted.
Abstract Title:
The Prevalence of Human Enteric Viruses and Protozoa in Non-Traditional Agricultural Water

Primary Author Block:
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Abstract Body:
Background: With the global population expected to rise to 9.3 billion by 2050 the challenges facing agriculture are immense. Limitations in the availability of arable land and the need to increase crop yields per m² are substantial but perhaps a more significant predicament will be managing these challenges in the face of climate change, more specifically, water availability. It is essential to evaluate associated food safety risks to determine the feasibility of using alternative water sources. This study was performed to detect pathogenic protozoa and viruses in surface and recycled waters evaluated as potential alternative water sources for crop irrigation, including potential of enteric viruses to serve as fecal indicators in non-traditional water sources. Methods: Water was collected on four dates from July through October (n=11), including three vegetable processing, four recycled, and four surface water samples. For Protozoa, 10-20L were filtered using an Envirochek® HV Capsule at a filtration rate of 2L/min. Filters were eluted according to EPA Method 1623. DNeasy Power Water Extraction Kit was used according to manufacturer’s custom instructions. qPCR was performed using QuantiNova Probe Assay Kit. For virus detection, 20-40 L of each water sample was processed using positively charged NanoCeram filters which were eluted using sodium polyphosphate buffer followed by concentration using 100 kDa Centricon filters. Viral DNA and RNA was extracted using the Allprep PowerViral DNA/RNA kit and analyzed by qPCR for DNA viruses and RT-qPCR for RNA viruses. Results: Samples were assessed for human viruses and protozoa. Overall for Cryptosporidium parvum, 54% (n=6) of water samples tested positive; including 75% (n=3) of wastewater and 50% (n=2) of surface water samples tested positive. Overall for Cyclospora cayentenesis, 72% (n=8) of the water samples tested positive; where all recycled and surface water samples tested positive (n=8). Interference in sample processing by qPCR was more notable in virus analysis, where with hepatitis A virus the CT values of the samples were significantly higher than those of the controls and were therefore excluded. Adenovirus 40 and 41 were detected in 18% of water samples, including 1 surface and 1 recycled water sample. Conclusions: Understanding the prevalence of enteric pathogens in alternative agricultural waters will enable the establishment of on-farm solutions and subsequent safe use of these waters in irrigation to reduce impending agricultural water challenges resulting from climate change.
Elucidating the Long-Term Impact of Disinfection Strategies on the Drinking Water Microbiome

Primary Author Block:
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Abstract Title:
Elucidating the Long-Term Impact of Disinfection Strategies on the Drinking Water Microbiome

Abstract Body:
Background: Disinfection is the common approach for controlling microbial growth in drinking water systems and is routine practice in USA, UK, etc. In contrast, some countries in western Europe, (e.g. Netherland), provide consumers with drinking water free from any disinfectants (1, 2). The contrast in microbial control strategies between these systems provide an ideal opportunity to investigate long-term impacts of disinfection on the drinking water microbiome. In this study, we aim to utilize genome-level information to identify microbial metabolic traits under strong selection in both types of systems. We are particularly focused on candidate phyla radiation (CPR) (3), which are highly prevalent in drinking water systems and their characterization may provide insights into the minimal metabolic capacity required to survive in oligotrophic drinking water systems. Methods: Samples were collected from the 15 drinking water systems in Netherlands, England, Wales, and Scotland. A range of chemical parameters were analyzed for each sample. Microbed were harvested from the samples by filtering through 0.22 μm Sterivex filters. DNA was extracted directly from the filters, prepared for sequencing using Nextera XT kit, and sequenced on Illumina HiSeq 2500 to obtain 250-bp paired-end reads. Reads were quality trimmed (4) and assembled using MetaSPAdes (5). Coverage information was generated by mapping reads against the generated scaffolds longer than 500bp. Scaffolds longer than 2500bp were clustered into metagenome assembled genomes (MAGs) using CONCOCT(6), and the completeness of output results were validated by applying CheckM (). Anvi’o (8) was used curate MAGs manual curation and phylogenetic placement. Results and Conclusions: More than five million scaffolds longer than 500bp were generated which accounted for 67-99% of the all reads from each sampling location. Composition and coverage based binning resulted in nearly 700 MAGs, with 261 high quality MAGs. Of these, 140 and 121 MAGs are derived from system with and without a disinfectant residual. We are in the process of curating these MAGs which will be followed by determination of SAAV/SNV ratio for each MAG. This will be followed by determining the level of selective stress in system, and the metabolic pathways of organisms’ subject to high selective stress will be reconstructed. In addition, we will also identify core metabolic traits shared in both disinfected and non-disinfected systems and compare dispensable genomes which are unique to these different systems.
Abstract Title:
Enumeration of Coliphages in Urban Polluted Rivers and Hosp. Wastewaters in Addis Ababa, Ethiopia

Primary Author Block:

Abstract Body:
Background: The monitoring of river water and wastewater for fecal pollution is becoming increasingly significant as world's population become more urbanized. In order to reduce the risk of disease in public health, good fecal indicators are required. Conventionally, bacterial indicators have been used as microbial indicators to monitor fecal pollution in waters. Nevertheless, it has been recommended that these bacterial indicators are not good for predicting enteric viruses. Methods and Materials: A cross sectional study was carried out on 94 samples of 32 urban polluted urban rivers in 10 sub-cities of Addis Ababa and 30 samples of the cities' four hospitals wastewaters at Ethiopian Public Health Institute between February and April, 2017. For all the samples, about 150 ml samples were collected into sterilized 200 ml glass bottles and transported on ice to the laboratory. The samples were maintained at 4°C. Microbiological investigations were done within 24 hours after collection. The simultaneous detection of both types of male-specific and somatic Coliphages from the samples were done using Escherichia coli CB390 as the host bacterium according to the single agar layer plaque assay. The Host log phase containing 0.15% ampicillin was applied with magnesium chloride in double strength tryptic soy agar [Difco]. The phages were incubated for 16-24 hours at 37oc and the plaques were enumerated. The coliphage enumerated was computed per 100 mL of the sample. Result: Of the total 124 samples tested from waters in Addis Ababa, total coliphages were detected in 47(37.9%) samples. Total coliphages enumerations ranged from <1pfu/100ml to 5.2×10³ pfu/100ml for urban polluted rivers and <1pfu/100ml to 4.92×10³ pfu/100ml for hospitals wastewaters. Of 94 polluted urban rivers samples in capital city of Ethiopia, total coliphages were observed in 22 of 32 rivers and 44 (46.8 %) samples in nine of 10 sub-cities. Of the total hospitals wastewaters samples, total coliphages were noticed in half of the hospitals in 3(10%) samples. P-values for coliphages using Kruskall-Wallis test for the samples by sample type were 0.001. Conclusion: The detections of total coliphages in this research suggest the possibility that polluted urban rivers and Hospital wastewaters may be a source for pathogenic viral infections. Unless coliphages, viral and fecal indicators are assessed in rivers and hospital wastewaters by public health agencies, waterborne infections cause a major risk to public health.
Background: Coastal estuaries and bays are complex in both nature and anthropogenic gradients, posing intensive stress for the microbial communities inhabited there, which provide fundamental eco-services such as nutrient cycling and pollutants degradation. However, it remains a question how coastal microbial diversity and function are changed by anthropogenic disturbances besides intrinsic strong natural spatiotemporal environmental dynamics.

Methods: We collected sediment samples from Hangzhou Bay, one of the most severely polluted bays along China’s coast, covering typical land-sea gradient in two years. Seawater qualities and sediment physicochemical properties were measured, and the microbial taxonomic and functional structures were explored by using 16S rRNA gene sequencing and shotgun metagenomic sequencing, respectively.

Results: The results indicated significant bacterial taxonomic variations along the space and between the 2 sampling years, even though no significant environmental changes were observed between the years. However, the communities’ function only slightly varied spatially and kept steady between the years. Compared with geographical distance, changes in environmental factors, primarily seawater salinity, dissolved inorganic nitrogen (DIN), total phosphorous (TP), and sediment nitrate and sulfate, were more significantly correlated with community taxonomic and functional dynamics. Nevertheless, the community taxonomic structures were more sensitive in responding to seawater pollutants (DIN and TP) than their functions. Network analysis identified distinct modules that composed with different taxonomic groups. The relative abundance for different modules exhibited disparate dynamic patterns spatially. Also, individual module responded differently to environmental factors, including seawater pollutants and factors indicating natural land-sea gradients (salinity, depth, etc.), implying differentiation in ecological niches for them.

Conclusions: In this study, we explored both taxonomic and functional dynamics of sediment microbial communities, and elucidated how environmental factors were correlated with communities’ changes. Our results indicated that the sediment microbial communities were sensitively responded to environmental changes, whereas their functions were more stable. The human induced seawater pollutants played comparable role in microbial community dynamics along with natural land-sea gradient in anthropogenically disturbed coastal area.
Abstract Title:
Annual Changes in the Sediment Microbiome Structure During Dev. of A Constructed Wetland
Primary Author Block:
M. Izadmehr, K. Rockne; Univ. of Illinois at Chicago (UIC), Chicago, IL
Abstract Body:
Hypoxia in the Gulf of Mexico results from high nutrient loading from the midwestern USA through the
Mississippi River. Illinois is one of the largest contributors of both N and P loading to the Gulf. IL has
pledged to reduce agricultural nutrient releases to combat this environmental issue. We are
constructing wetlands to capture drain tile from corn/soybean cropland to capture and treat tile
drainage to stimulate microbial denitrification as a targeted response to this major source of agricultural
N export. Denitrification of NO3- to N2 and N2O is the most important nutrient removal processes in a
constructed wetland. This process is carried out primarily by denitrifying bacteria, but this biochemical
capability also has been found in archaea and fungi as well. Most denitrifiers in wetlands are found in
the top layer of the sediment, where there is a balance between electron donor (fermentation products
from the organic matter’s breakdown) and electron acceptor (NO3-). While denitrification assays have
been used for decades to understand the capacity for nitrate removal in sub-samples, this technique
does not tell us about the state of the underlying denitrifying microbial community structure. More
specific information on this activity can come from qPCR of the denitrifying genes nirS and nirK that
catalyze the reaction NO2-àNO, and nosZ that catalyzes the reaction N2OàN2. Segmented sediment core
samples were taken from nine locations in the first wetland (W1) constructed in fall 2015 on an
approximate monthly basis. These samples were characterized for organic matter, spatial and temporal
qPCR of the denitrifying genes nirS, nirK and nosZ over the monitoring period. Different sampling dates
have been chosen to cover all four seasons, initial conditions in the field, and months with high nitrate
removal. The performance of wetland W1 has been monitored since Spring 2016, which removed 12%
and 35% of influent NO3- in 2016 and 2017, respectively, with very high removal (70-80%) observed in
summer 2017. The abundancy of denitrifying genes will help us better understand how incomplete
denitrification, resulting in nitric oxide production (nirS and nirK) and complete denitrification process
(nosZ) varies temporally, spatially (at depth and across the wetland), as well as with other
physiochemical characteristics of the sediment and water column and nutrient removal rate. These
results will help us determine what limits denitrification rates in the wetland.
Abstract Title: Characterization of the Microbial Community Driving On-Site Wastewater Treatment in Nitrogen Removing Biofilters (NRBs)

Primary Author Block:
K. Langlois, J. L. Collier, N. Volkenborn, X. Mao, M. Graffam; Stony Brook Univ., Stony Brook, NY

Abstract Body:
Innovative on-site wastewater treatment systems (OWTS) are a promising solution to problems caused by excessive fixed nitrogen in areas without sewers and wastewater treatment plants. Passive nitrogen-removing biofilters (NRBs) are partially engineered OWTS capitalizing on natural microbial processes to remove nitrogen from wastewater. Wastewater is evenly dispersed to NRBs, built under homeowners’ yards, after settling in a septic tank. The hypothesis is that physical characteristics of NRB design affect microbial community structure, promoting the desired processes. The top sandy layer provides oxic conditions with little labile organic carbon to promote nitrification, and the bottom sand-lignocellulose (sand-lc) layer provides anoxic conditions with a long-lasting organic carbon source to promote denitrification. This hypothesis predicts that microbial assemblages in the sand and sand-lc layers will be distinct from each other and that the differences between them will be related to differences in environmental conditions, such as oxygen availability. To test these predictions, aged matrix from a full-scale test NRB was repacked into lab-scale microcosms simulating NRB structure and exposed to artificial wastewater for two weeks. Planar optodes served to document oxygen availability within each microcosm. Matrix samples were collected from consistently oxic (ox.), anoxic (anox.), and oscillatory (osc.) zones. The microbial community was characterized by Illumina high-throughput sequencing of 16S rRNA V3/V4 gene amplicons. Sequences were grouped by QIIME into operational taxonomic units (OTUs) at 97% identity for further analysis. The anox. zone matrix contained ~1000X more 16S rRNA gene copies per milliliter than the ox. zone, average 4.08E8 in anox. and 2.95E5 in ox. Principal coordinate analysis, using the weighted UniFrac metric, showed three distinct groups of samples: ox., osc., and anox. Groups were consistent with mean time oxic measured in the three zones. This pattern was also observed in an indicator species analysis and nonmetric multidimensional scaling ordination using the Bray-Curtis metric. In the NMS, oxygen availability strongly correlated to both axes. These results support the hypothesis that NRBs’ physical characteristics and oxygen availability patterns result in different microbial assemblages in the two layers. The presentation will explore which taxa drive the differences between the ox., osc., and anox. zones in NRBs and the insight it provides into NRB function.
Abstract Title:
Shifts in the Activated Sludge Microbiome and Nitrogen Cycling Genes During Low Temperature Seasonal Nitrification Failure

Primary Author Block:
J. Johnston, T. LaPara, S. Behrens; Univ. of Minnesota, Minneapolis, MN

Abstract Body:
Background: Wastewater treatment plants frequently undergo seasonal nitrification failure due to cold temperatures yet maintain removal efficiency for other contaminants. We hypothesized that we could correlate temperature shift to nitrification failure by seasonally monitoring the nitrogen cycling community. Methods: We monitored three nearly identical sequencing batch reactors over the course of a year with respect to performance, microbial community composition via 16S rRNA gene amplicon sequencing, and nitrogen cycling gene abundance using qPCR. Results: Our three reactors demonstrated significantly similar changes to their core microbiome, and only subtle variations with seasonal and transient OTUs. We quantified a lag in recovery after nitrification failure which required reactors to be at 1.73°C warmer in the spring to reach similar performance observed in the fall. Despite the change in nitrification performance, all nitrogen cycling gene abundances remained constant, as did the family of Nitrosomonadaceae. There were fluctuations between the genus Nitrosomonas, and other members of the family Nitrosomonadaceae. This suggests that there are differences in kinetics between nitrifying organisms, or that the changes in performance result from another organism, potentially an uncultured Saprospiraceae. Conclusions: This research ultimately strengthens the understanding of the core microbial community in activated sludge by revealing the cyclic nature of the community throughout the seasons, and expands on shifts during nitrification failure in the winter.
Enrichment and Isolation of Complete Ammonia-Oxidizing Bacteria from Full-Scale Wastewater Treatment Plants

K. J. Vilardi, I. Cotto, A. J. Pinto; Northeastern Univ., Boston, MA

Traditionally, aerobic nitrification was thought to be driven by a two-step process utilizing both strict ammonia-oxidizing microbes (AOM) and nitrite-oxidizing bacteria (NOB). However, the fundamentals of nitrification have drastically changed with the discovery of a single microorganism which can completely oxidize ammonia to nitrate, i.e. complete ammonia-oxidizing bacteria (comammox - CMX). CMX bacteria could significantly influence the nitrogen removal processes in terms of design, cost, and resource recovery for the wastewater industry. Complete nitrification yields more energy than either of the single steps making it a more energetically favorable process (Daims 2015). Therefore, potentially giving CMX bacteria an advantage over AOM and NOB could offer extreme value towards enhancing the nitrogen removal process for wastewater treatment. However, more investigations into their ecology and physiology are critical to enable this process development. The characterization of comammox (i.e. abundance, diversity, growth rate, etc.) will be elucidated through wastewater sample enrichment and kinetics assays. Samples were obtained from a variety of treatment plants encompassing different treatment trains and nitrogen removal configurations. Samples from full-scale systems were screened for the presence of CMX bacteria using recently published primer sets to select a subset of samples for CMX bacterial enrichment. Enrichments were started with biomass from select systems in ammonia oxidizing media with varying levels of ammonia supplementation. Ammonia consumption and concomitant nitrite and nitrate production was monitored. Currently, real-time PCR is being used to track the nitrifier population dynamics in these enrichments. The establishment of a dominant CMX population will be followed by kinetic assays to measure their extant and intrinsic ammonia and nitrite oxidation rates. Further, we intend to couple short and long-read metagenomics and transcriptomic sequencing of initial inoculum and enrichments at varying time points to obtain metagenome assembled genomes of nitrifying populations in the enrichment with particular focus on CMX bacteria. Coupling in situ kinetic measurements with metatranscriptomics and metagenomics will enable us to systematically investigate the eco-physiology of CMX bacteria; this would be an important step towards their application in engineered water systems.
Abstract Title:
Bacterial Community of Pilot Plant-Scale Biol. Treatment Sys. and its Nitrogen and Phosphorus Removal of Marine Wastewater
Primary Author Block:
J. Kim, S. Kang, H. Kim, S. Jeong, S. Kim, S.-S. Lee; Kyonggi Univ., Suwon, Korea, Republic of
Abstract Body:
Background: Saline wastewater in high concentration is difficult to be biologically treat. It is helpful to reduce cause of the red-tide in the long-term. However, it is required for eco-friendly and effective ways. This study is to apply the pilot plant-scale effective biological treatment of high salinity effluents from land-based fish farm and trace bacterial community for operation period. Methods: Bacillus sp. KGN1 (KEMB 3401-006) and Vibrio sp. KGP1 (KEMB 3001-129) was revealed their nitrogen and phosphate removal efficiency (R.E.) in the batch culture as well as the lab-scale sequencing batch reactor (SBR) in the previous study. Moreover, these bacterial strains and marine sediment was adapted as an eco-friendly marine sludge for the application of biological treatment system. The pilot plant-scale biological treatment consists of an influent tank in 2.5 m3, an SBR reactor (involving the aeration equipment, the mixture, and the decanter) in 4.5 m3 and an effluent tank in 2.5 m3 with auto-control system. Results: Using the pilot plant-scale SBR system with eco-friendly marine sludge from the land-based fish farm improved the treatment performance as indicated by averagely 69.8% R.E. of CODCr (Influence: 129.1 mg/L, effluence: 39.0 mg/L), 92.7% R.E. of NH3-N (influence: 5.4 mg/L, effluence: 0.4 mg/L), 73.1% R.E. of T-N (influence: 7.8 mg/L, effluence: 2.1 mg/L), 62.5% R.E. of T-P (influence: 1.6 mg/L, effluence: 0.6 mg/L) at the optimal operation conditions (4h/cycle; 10 min influent period, 2 h 50 min aeration period, 0.5 h settlement, 0.5 h idle& effluent; hydraulic retention time (HRT), 6h; solids retention time (SRT), 12 d). Bacterial community in the eco-friendly marine sludge of the SBR tank was analyzed during operation period. The analytical results of the microbial community showed that eco-friendly marine sludge contained predominant bacteria genera Sulfurovum (36.3%), Vibrio (17.6%), Sulfitobacter (12.6%), Oceanisphaera (9.2%) and Cobetia (4.5%) in the optimal condition of the summer season, while genera Psychromonas (45.6%), Vibrio (13.3%), Psychroserpens (4.3%), Gatbulibacter (5.7%) and Family Oceanospirillaceae (8.3%), Rhodobacteraceae (3.4%) in the optimal condition of in the winter season. Conclusions: The study proposed that analysis of the bacterial community is one of the effective microbial strategy for maintaining and operation of biological treatment from marine wastewater.
Monitoring the Potential Environmental Impacts of Aerobic Sewer Systems

Primary Author Block:
McNeese State Univ., Lake Charles, LA

Abstract Body:
Background: Aerobic sewer systems are used to treat domestic wastewater in many rural areas of the United States. The effluent from many of these systems is discharged directly into disposal ditches, which rely on sunlight for disinfection purposes. In spite of this practice, very few studies have examined the microbial quality of effluent from these systems in order to better understand their potential environmental impacts. The goal of this work was to assess the potential environmental impacts of aerobic sewer system effluent by: 1) Quantifying E. coli, fecal coliforms, and heterotrophic bacteria in effluent samples that were collected from aerobic sewer systems and 2) Comparing numbers of E. coli, fecal coliforms, and heterotrophic bacteria in ditches that received aerobic sewer system effluent with water samples from non-sewage impacted sources. Methods: The concentrations of E. coli and fecal coliforms in aerobic sewer system effluent, ditches that received aerobic sewer system effluent, and water samples from non-sewage impacted sources were monitored in triplicate using pour plate assays. These pour plate assays were performed using CHROMagar ECC medium incubated at 44°C for 24 hours. Heterotrophs were enumerated in these same samples using three-tube most probable number (MPN) dilutions. All of these MPN dilutions were performed in half strength tryptic soy broth (TSB) incubated at 37°C for 24 hours. Results: The numbers of E. coli, fecal coliforms, and heterotrophic bacteria in the effluent samples from aerobic sewer systems ranged from BDL-7.3 x 107 CFU/100 ml, 1.1 x 105-1.4 x 108 CFU/100 ml, and 1.1 x 105-1.1 x 109 MPN/100 ml, respectively. The numbers of E. coli, fecal coliforms, and heterotrophic bacteria in aerobic sewer system effluent, ditches that received aerobic sewer system effluent, and water samples from non-sewage impacted sources were monitored in triplicate using pour plate assays. These pour plate assays were performed using CHROMagar ECC medium incubated at 44°C for 24 hours. Heterotrophs were enumerated in these same samples using three-tube most probable number (MPN) dilutions. All of these MPN dilutions were performed in half strength tryptic soy broth (TSB) incubated at 37°C for 24 hours. Results: The numbers of E. coli, fecal coliforms, and heterotrophic bacteria in the effluent samples from aerobic sewer systems ranged from BDL-7.3 x 107 CFU/100 ml, 1.1 x 105-1.4 x 108 CFU/100 ml, and 1.1 x 105-1.1 x 109 MPN/100 ml, respectively. The numbers of E. coli, fecal coliforms, and heterotrophic bacteria in disposal ditches that received aerobic septic systems effluent ranged from BDL-8.35 X 104 CFU/100 ml, 1.3 x 104-greater than 1.5 x 106CFU/100 ml, and 2.1 x 106-1.1 x 109 MPN/100 ml, respectively. E. coli concentrations were BDL in all non-sewage impacted waters, while fecal coliform and heterotroph concentrations ranged from BDL-2.4 x 105 CFU/100 ml and 9.33 x 105-9.33 x 107 MPN/100 ml, respectively. Conclusions: This study shows that aerobic sewer systems often release large numbers of E. coli, fecal coliforms, and heterotrophic bacteria into the environment. The observation of higher numbers of E. coli, fecal coliforms, and heterotrophic bacteria in disposal ditches that receive aerobic sewer system effluent relative to non-sewage impacted water sources suggests that these systems are negatively impacting the environment.
Abstract Title:
Wastewater Treatment Plant Effluent Indicators for Assessing Effluent Dominated Waters
Primary Author Block:
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Abstract Body:
Studies have suggested human associated genetic markers (i.e. HF183/286R and Lachno3) are useful tracers of untreated sewage and potentially treated wastewater effluent. However, these human genetic markers are present in both treated and untreated wastewater, albeit at very different concentrations, and it is difficult to discern between a small amount of sewage and large amounts of treated effluent in impacted surface waters. To address this problem, we first characterized the human associated marker loads in wastewater influent (untreated sewage) and treated wastewater effluent in a single wastewater treatment plant over a two-year period. Results have shown that human associated markers are in high abundance in influent (Lachno3 median 3.53x10^7 Copy Number (CN)/100 mL, range 2.06x10^7 - 8.16x10^7 CN/100 mL; HF183/286R median 3.94x10^7 CN/100 mL, range 2.12x10^7 – 9.83x10^7 CN/100 mL) and present at variable but abundant loads in wastewater effluent (Lachno3 median 9.62x10^4 CN/100 mL, range 6.08x10^3 - 2.25x10^6 CN/100 mL; HF183 median 2.25x10^6 CN/100 mL, range 1.51x10^4 - 8.95x10^6 CN/100 mL). Results indicate wastewater influent and effluent both contain human associated markers Lachno3 and HF183 at relatively elevated abundances, making effluent indistinguishable from influent. Consequently, we sequenced wastewater influent and effluent to identify sequence types within specific taxa which can be used to track treated effluent in the environment separate from raw sewage (influent). Comparison of whole microbial community sequencing (Illumina MiSeq, 16S rDNA) indicated a core group of bacteria that could be potential markers which offer an improved method to track sewage and wastewater effluent separately in aquatic ecosystems. This work demonstrates the utility of examining the microbial community of treated wastewater effluent to help separate effluent from potential confounding sources nearby, such as conveyance systems and septic tanks, which may be contributing to decreased water quality nearby.
Kinetic and Microbiological Characterization of Varying Size Fractions of Aerobic Granular Sludge

Primary Author Block:
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Abstract Body:
Half saturation coefficient (Ks), which is defined as the substrate concentration at which the kinetic rate equals half of the maximum specific growth rate, is a key factor for the performance of biological wastewater treatment systems. As in other biological models, Ks values in wastewater are ruled by diverse factors such as microbial community structure, molecular diffusion, and substrate availability. Aerobic granular sludge (AGS) is classified as a diffusion limited system that exhibits a dissimilar limitation pattern due to the presence of different sizes of granules. The goal of this study is to determine the ammonia, nitrite, and oxygen Ks_{app} in granular size fractions and to present an assessment of the microbial community dynamics in granular sludge. To achieve this Ks_{app} for ammonia, nitrite, and oxygen were determined for each granule size. Microbial community composition and structure of each size fraction was described using Fluorescent in situ hybridization (FISH) and qPCR. In general, the obtained data suggest that the affinity of Ammonia Oxidizing Bacteria (AerAOB), Nitrite Oxidizing Bacteria (NOB), and heterotrophic bacteria increases with increased granule size. Apparent Ks values for ammonia (K_{NH3,app}) values were in the range of 11.63 to 35.18 mg NH3-N /L whereas the KNO2_{app} values ranged between 1.18 to 16.71 mg NO2-N/L. This is likely due to the AerAOB and NOB presence being confined to the outer granule layer, whereas the bulk VSS is composed by other organisms. SOUR values, exhibited a similar trend with granule size as the apparent Ks. When COD was used as substrate for the evaluation of maximum activity of heterotrophic biomass, the activity decreased with increasing granule size. The results presented in this study agree with previous studies that have demonstrated the influence of mass transfer related to particle size in the kinetics of nitrifiers. As the surface to volume ratio increases with smaller granules, the relative proportion of the biomass that is aerobic, heterotrophic or nitrifying, increases. This mass transfer limitation changes the microbial community structure, and together, these factors are responsible for decreased SOUR values with increased size. In line with our main hypothesis, Ks_{app} increased with increasing granule size. However, this study also suggests that at higher particle size these trends can be influenced by differences in microbial composition and distribution. Ks values defined in this work can be incorporated into models to simulate the performance of reactors for nitrification.
Abstract Title:
Metagenomic Characterization of Microorganisms in An Advanced Water Treatment Facility

Primary Author Block:
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Abstract Body:
Background: Increasing demand for potable water in many communities has led to the reuse of treated municipal wastewater as a new, renewable supply of potable water. The Ground Water Replenishment System (GWRS), in California, is the largest advanced water purification facility (AWPF), treating up to 100 million gallons per day of secondary treated wastewater to produce potable quality water by using a three step advanced treatment process: microfiltration (MF), reverse osmosis (RO), and ultraviolet light with hydrogen peroxide. Aside from indicator organisms, identification of microbial communities and of pathogens at different levels of advanced treatment has been problematic. Lack of accurate and sensitive identification methods is one important reason for this knowledge gap. Advancement of next-generation sequencing and development of ultrafast bioinformatic tools proved to be a very powerful and unbiased method that is currently being used to identify microorganisms. Metagenomic sequencing of total DNA and RNA combined with CosmosID bioinformatics was used to identify microorganisms, functional genes and to characterize microbial communities in biofilms from MF and RO membranes. Secondary treated influent (Q1), to the GWRS treatment train was also characterized. Methods: Total DNA and RNA are extracted from biofilm scrapings from MF and RO membranes and from influent water (Q1). Samples are sequenced by Illumina HiSeq 400 and analyzed by CosmosID bioinformatics software. Results: Fungi, parasites, bacteria, viruses, and antibiotic resistance genes (ARGs) are identified in all samples. Bacteria considered opportunistic pathogens comprised a portion of the microbial signatures in all samples. The greatest bacterial species diversity was identified in the influent, Q1; the least number of bacterial species were identified in the RO-biofilm. Approximately 1,864 bacterial species were identified in Q1, fewer (1,445) in the MF-biofilm, the least (n=821) identified in the RO-biofilm. Similar patterns were observed with fungi, parasite, viruses and ARGs, revealing progressively lower abundance of species and functional genes in the MF and RO-biofilms. Pepper mild motile virus (PMMoV) and E. coli bacteriophage were not identified in the RO-biofilms. Conclusion: Metagenomic sequencing of DNA and RNA is a versatile and accurate method for identifying and characterizing microbial communities in MF, RO-biofilms, and in treated wastewater.
Abstract Title:
Ozone Disinfection of E. coli in Secondary Treated Final Effluent Wastewater

Primary Author Block:
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Abstract Body:
“Disinfection is considered a primary mechanism for the inactivation/destruction of pathogenic organisms to prevent the spread of waterborne diseases to downstream users and the environment (1). Most diarrheal deaths in the world are caused by unsafe water, sanitation or hygiene (2).” A point of use treatment method has been developed for drinking water using ozone as a disinfectant. The aim of this research was to determine how effective ozone disinfection treatment would be in the destruction of E. coli. The relevance of this research is to develop a protocol for safe effective disinfection treatment. Ozonation disinfection was used on secondary treated effluent (FE) wastewater from the Metropolitan Water Reclamation District of Greater Chicago. FE under goes some treatments but not disinfection. Ozonation disinfection was performed with added humic acid to replicate turbidity from 5-35 NTU in 100L samples for 9 trials. Turbidity influences the effectiveness of drinking water disinfection. The data shows rates of disinfection in FE without added turbidity may reach disinfection level of 0 MPN E. coli present at +/- 60-120 minutes. Disinfection in FE with added humic acid show disinfection occurring at much slower rate, +/- 0-100 MPN E. coli present 120-180 minutes. The rate equation (<u>concentration E. coli</u> + <u>turbidity</u>) x (<u>time</u>) determines the effectiveness of disinfection. Higher concentrations of turbidity suggest more time necessary to reach safe levels of disinfection. The impact of these results show that more effective methods/treatments are needed to help remove/decrease levels of turbidity in drinking water disinfection if ozonation is to become an effective tool in producing safe drinking water. The impact to the field of microbiology is the effectiveness of ozonation in inactivation/destruction of E. coli. <br/>

"Disinfection is considered a primary mechanism for the inactivation/destruction of pathogenic organisms to prevent the spread of waterborne diseases to downstream users and the environment (1). Most diarrheal deaths in the world are caused by unsafe water, sanitation or hygiene (2)."
Abstract Title:
Two Wastewater Treatment Plant Effluents As Sources of Pathogenic Vibrio Species to their Receiving Water Bodies.

Primary Author Block:
O. Gcilitshana; Univ. of Fort Hare, Alice, South Africa

Abstract Body:
Background: Despite the advances and developments such as improved sanitation and safe water in some developing countries, acute microbial diseases still thrive and continue to distress millions of people (Ali et al., 2015). In South Africa, a significant proportion of rural community residence still uses untreated river water for drinking and other direct uses, and the area under study has had a serious diarrheal outbreak, hence the need to research on the potential cause of these cholera associated outbreaks manifest in the area. This study was conducted to assess the occurrence of cholera-causing pathogens in final effluents and surface waters of the Eastern Cape Province of South Africa. Methods: Two wastewater treatment plants and their discharge points in the Eastern Cape Province of South Africa were selected for this study. The two wastewater treatment plants serve as feeders of a large river tributary that has its water directly used for domestic use and irrigation of farms in the area. For this study, nine samples (final effluents, discharge points, upstream and downstream of their receiving waterbodies) were collected monthly for a period of 12 months (December 2016 - November 2017). Physicochemical parameters that influence the presence of vibrio pathogens were measured on site using multi-parametres, while vibrio densities were measured using membrane filtration method on TCBS agar and further confirmed by molecular techniques (conventional PCR). Confirmed vibrio isolates were further delineated into V. parahaemolyticus, V. vulnificus, V. alginolyticus and V. fluvialis pathotypes. Results: Most of the physicochemical parameters measured (pH, turbidity, temperature, salinity etc) did not comply with most recommended standards for drinking water. Furthermore, there was correlation (p > 0.05) of these with the vibrio counts. The counts of Vibrio spp. varied with months in all the study sites ranging between 101 and 104 CFU/100mL. Vibrio distribution also showed seasonality with high counts being obtained in autumn (p < 0.05). So far, 453 isolates have been confirmed as Vibrio spp. with only 12 (6%) belonging to the V.cholerae, and 8 (4%) belonging to the V.mimicus. Conclusions: Even though there is high detection of vibrio spp. and notable correlation between physicochemical parameters measured and the distribution of vibrio pathogens, the work is still ongoing and therefore, no conclusions can be made as yet.
Characterization of Biomass Degrading Bacillus and Paenibacillus Species from A Wet Fermentation Digester

Primary Author Block:

Abstract Body:
The microbial communities of digesters fuel the breakdown of agricultural and food wastes, generating usable energy in the form of methane and yielding high-nutrient fertilizers. However, the composition and metabolic functions of these communities remains under-explored. Biodigesters provide unique environments for finding microbes with novel biomass degrading phenotypes that could provide a source of enzymes usable for downstream industrial applications. Our research explored the microbial diversity and function of a wet fermentation digester (Allen Farms, Wisconsin). The Allen Farm system consists of a two-stage mixed-plug flow digester operating in the mesophilic temperature zone with a total capacity of 302,833 liters. It operates with an organic loading rate of 4.5 kg VS/m3/day, yielding 31 m3/hr of biogas at a mean methane concentration of 58%. The organic inputs consist of manure scrape, used dairy cattle bedpack, and industrial food wastes to boost production. A snapshot of the microbial community in the biodigester was determined with high throughput sequencing. The metagenome was analyzed for biomass-degrading bacterial taxa. Aerobic bacteria were isolated from feedstock on various cellulose derivatives. Six isolates were characterized for biomass degradation utilizing grain-based substrates, carboxymethyl cellulose, cellobiose, and xylan. The microbial community was highly diverse, dominated by Firmicutes (45.9%), Bacteroidetes (26.6%), Proteobacteria (9%), and Actinobacteria (6.5%), resembling bacterial composition of the bovine gastrointestinal tract. Isolates belonged to the genera of Bacillus and Paenibacillus, were reasonably represented within the Firmicutes sequences. These strains produced high levels of extracellular enzymes when cultured on unprocessed grains (e.g. oats and barley). Extracellular enzyme production was enhanced at mesophilic conditions, much like the habitat of the digester. Strains preferred corn xylan compared to beechwood xylan, producing more extracellular enzymes and greater activity to release xylose monomers. As xylan is a dominant polymer of hemicellulose, extracellular enzyme production and degradation of xylan are important for digester efficiency. Xylan breakdown was an observed common phenotype of these specific Bacillus and Paenibacillus isolates. These findings suggest that Bacillus and Paenibacillus are ideal models for novel enzyme discovery.
Abstract Title:
Challenge to the Efficient Anaerobic Wastewater Treatment by Enhancing Extracellular Electron Transfer

Primary Author Block:
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Abstract Body:
Background: It is significantly important to reduce social total energy related to reduce CO2. From these viewpoints, the development of efficient anaerobic wastewater treatment (ANWT) is indispensable.

Methods: Wastewater treatment reactors (2 L) were constructed under aerobic condition (control) and anaerobic conditions equipped with electrode (AE) or electrode including a conductive bio-mineral (AEBM). External resistance was 51 Ω and carbon paper electroplated with platinum (0.5 mg Pt cm-2) was used as cathode (1 cm2). The bio-mineral was produced by sulfate-reducing bacterium and had a rechargeable property. These reactors were enriched by circulating batch culture. Fresh artificial wastewater at COD of 600 ppm was exchanged with half volume of supernatant every 2 days in the control reactor and every 10 days in the AE and AEBM reactors. Initial amount of activated sludge was 1500 ppm. Results: In the control reactor, COD removal ratio was approximately over 90% every cycle and activated sludge increased to about 3000 ppm in 22 days. The COD removal ratio was over 80% in anaerobic reactors. The amount of activated sludge in the AE and AEBM reactors were maintained at approximately 1370 and 930 ppm during this experiment. The average rate of COD removal per amount of activated sludge in the AEBM was approximately 0.09 mg L-1 day-1 ppmMLSS-1, which was 1.9-fold and 1.5-fold of the control and the AE reactors. These results demonstrated that the conductive bio-mineral was useful for enhancing the anaerobic respiration, resulting in higher COD removal ratio. Microbial community structures were analyzed by DGGE-multidimensional scaling method. Dynamics of microbial populations in the AEBM reactor was different from the AE reactor. Especially, dynamics of planktonic populations in the AEBM was closer to anode population than the AE reactor. Conclusions: These results suggested that the bio-mineral affected not only biofilm but also planktonic communities through electronic flow, resulting in enhancement of anaerobic wastewater treatment activity. When we improve the treatment activity of AEBM to 10- to 30-fold, the AEBM would become practical technology.
Abstract Title:
Influence of Impervious Surfaces on Pathogen Occurrence and Human-Associated Bacteroides in An Urbanized Stream

Primary Author Block:
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Abstract Body:
Heavily urbanized watersheds are subject to many anthropogenic forces, ranging from surface storm run-off caused by impervious surfaces to sanitary and storm sewers, which can impact surface water quality through the addition of fecal indicator bacteria (FIB) and pathogens. In this study we determined if pathogen and toxin gene occurrence (Salmonella sp. and Shiga-toxin (Stx2)) or a human-associated Bacteroides marker (HF183MGB) could be explained by impervious surface cover in an urban watershed. Proctor Creek, a tributary to the Chattahoochee River located in Atlanta, Georgia U.S.A., is a highly urbanized watershed listed on the Environmental Protection Agency’s (EPA) 303(d) impaired waters list due to failing fecal coliform standards. The upper reach of this watershed originates in downtown Atlanta where impervious cover can reach 100% due to buildings, parking lots, and industrial sites, which results in an increased input of storm water runoff into the stream during heavy rains and contributes to flooding of residential areas. In contrast, the lower reach of the watershed contains significantly less impervious cover with land use that is dominated by forested and low density residential areas. Grab water samples were collected biweekly from November 2015 through July 2017 from twelve locations throughout the watershed (four in the upper reach and eight in the lower reach). Stx2 was detected at higher frequency in the upper (56% present) than the lower (37% present) reach. Salmonella sp., however, displayed the opposite trend and was detected more often in the lower reach (64% present) compared to the upper, more urbanized section (40% present). Similarly, the marker targeting human-associated Bacteroides, HF183MGB was also present throughout the watershed but was again higher in the upper (5.1 ± 0.1 log gene copies per 100 mL) than the lower (3.1 ± 0.1 log gene copies per 100 mL) reach. These data suggest that impervious surface area coverage might be predictive of the presence of certain markers and toxins (i.e. HF183MGB and Stx2) in the environment, while specific pathogens such as Salmonella sp. may need to be explained by other watershed stressors.
Abstract Title:
Outstanding Abstract Award: Stormflow Associated Taxa-Driven Functional Shifts in An Urban Stream Microbial Community

Primary Author Block:
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Abstract Body:
Urban streams are susceptible to stormwater and sewage inputs that can impact their ecological health and water quality. Microbial communities in streams play important functional roles and their composition and metabolic potential can help assess the ecological state and water quality. However, little is known about the response of urban stream microbiota to increased urban inputs resulting from rain events from a whole community composition and functional perspective. Here, we examined the microbial community composition and diversity in an urban stream part of the Chicago Area Waterway System during dry and wet weather conditions with both 16S rRNA gene sequencing and shotgun metagenomics. Metagenomics was used to assess population-level dynamics as well as shifts in the microbial community taxonomic profile and functional potential associated with substantial rainfall. Results highlighted significant influence of increased effluent flow following rain in shifting the stream microbial community from abundant freshwater taxa such as Actinobacteria and Pelagibacteraceae to those more associated with urban/anthropogenic settings such as Legionella and Arcobacter, with the introduction of exogenous organisms to the system likely a significant driver of the observed change. Shifts in the taxonomic composition were also linked to changes in functional gene content, particularly for transmembrane transport and organic substance biosynthesis. The after rain microbial community also harbored a higher relative abundance of antibiotic resistance genes, as well as genes encoding degradation of organic pollutants such as nicotine, phenol and 1,4-dichlorobenzene. Overall, this study provided evidence of stormflow impacts on an urban stream microbiome from an environmental and public health perspective.
Abstract Title: Characterization of Vibrio Species Isolated from Coastal Waters of Qatar
Primary Author Block: 
R. Fotedar1, A. Al Malaki1, A. Anand1, F. Ramadan1, S. Rashed2, K. Brumfield3, E. Ibrahim4, M. Al Marr1, R. R. Colwell5, A. Haq6, R. Fotedar1; 1Ministry of Municipality and Environment, Doha, Qatar, 2Univ. of Maryland, Maryland, MD, 3Maryland Pathogen Res. Inst., Univ. of Maryland, Coll. Park, MD, Maryland, MD, 4Hamad Med. Ctr., Doha, Doha, Qatar, 5Maryland Pathogen Res. Inst., Univ. of Maryland, Coll. Park, MD, Doha, MD, 6Maryland Pathogen Res. Inst., Maryland, MD

Abstract Body:
Background: The Vibrio genus is composed of motile, Gram-negative facultative anaerobes ubiquitous to marine and estuarine environments. While most interactions with microbial and multicellular hosts are commensal, some Vibrio’s have pathogenic tendencies. The genus consists of more than 80 identified species, of which 12 are considered to be human pathogens causing gastroenteritis and wound infections. Diarrhoea-causing non-cholera Vibrio’s have been commonly found in the warm, tropical water. This foundational report documents an ongoing study focused on the diversity and distribution of Vibrio species in the Arabian Gulf surrounding Qatar. Methods: A total of 1200 samples were collected from water, plankton, shellfish, algae, and sediment between January-October in 2017 from six coastal sites of Qatar. Additionally, 14 stool samples were collected from patients presenting with diarrhoea at the Hamad Hospital in Doha, Qatar. All the samples were enriched with APW and plated directly onto selective TCBS and CHROMagar, and incubated overnight at 37°C for isolation of Vibrio. Colonies of presumptive vibrios were selected and purified on Luria Bertani agar by repeat subculture. DNA was extracted from all the samples by boiling for 10 min at 99°C. Isolates were validated using molecular identification using PCR with primers specific to the genus Vibrio (567F-680R) and other Vibrio species specific primers. Results: Of 124 DNA examined by PCR, all samples were confirmed as Vibrio spp. of which 26 were confirmed to be V. parahaemolyticus, 40 to be V. alginolyticus, and 58 belonged to other Vibrio spp. Presence of V. cholerae was confirmed in 13 (93%) of stool samples. No V. cholerae or V. vulnificus, were detected in environmental samples. Discussion: V. alginolyticus and V. parahaemolyticus both have infectious potential in humans, and V. cholerae is the etiological agent of epidemic cholera—posing potential health concerns for the region. This investigation will continue to characterize the remaining environmental isolates including virulence-related genes, response to conventional antibiotics, for public health interest and routine monitoring these pathogens potentially will prevent Vibrio associated outbreaks in Qatar including the regional countries.
Abstract Title:
Fecal Indicator Bacteria, Microbial Source Tracking, and Pathogen Detection in A Polluted Hudson River Tributary

Primary Author Block:
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Abstract Body:
Long-term monitoring of fecal enterococci levels in the Hudson River and its tributaries have highlighted tributaries with consistently high levels of fecal contamination - with geometric means well above the recreational beach action value of 60 CFU/100-mL. This current study sought to characterize the animal sources of fecal pollution in the largest tributary with consistently high culturable enterococci levels via microbial source tracking (MST). The possible presence of human gastrointestinal pathogens in the watershed was also investigated via a high-throughput qPCR methodology. Water samples and water quality data were collected monthly from twenty-four sites in the tributary from May to October 2017. Culturable Enterococcus spp. and E. coli counts were obtained for each sample via Enterolert and Colilert, respectively. Four different qPCR MST assays were employed targeting markers of avian, bovine, equine, and human feces. An OpenArray qPCR panel was designed with 17 targets that included fecal indicator bacteria (FIB), MST targets, and 12 pathogenic bacteria, viruses, and protozoa. Water sample biomass was concentrated via tangential flow ultrafiltration and DNA was extracted prior to processing on the open array panel. Enterococcus spp. counts were consistently higher than E. coli counts, both via the culture-based measurements and via the open array qPCR panel measurements. MST data revealed frequent avian contamination in this watershed (in 86 out of 144 samples tested). Avian marker presence at a site was significantly correlated with higher levels of Enterococcus spp. and E. coli (p=0.002 and p=0.006, respectively, unpaired Wilcoxon Rank Sum test). Though human fecal contamination was detected less frequently across the watershed, it did correlate with higher levels of E. coli (p=0.029, unpaired Wilcoxon Rank Sum test). There were five sites where the human MST marker was detected for at least three out of six of the months tested. Of the human pathogens surveyed, a rotavirus strain was detected frequently across the sites surveyed. However, rotavirus concentrations were not correlated with the concentration of FIB in the watershed, suggesting different persistence and sources for FIB and rotavirus. The E. coli eae gene, human adenovirus, and Giardia lamblia were also detected with some regularity. This study demonstrated the utility of tying together culturable FIB data, microbial source tracking data, and pathogen detection to characterize fecal pollution in a freshwater watershed.
Abstract Title:
Trend Measurement of Pathogenic Organisms in Soil At the Vicinity of Ugwuaji Landfill Site, Enugu, Nigeria

Primary Author Block:
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Abstract Body:
The frequency of occurrences of pathogenic organisms in soil at the vicinity of Ugwuaji Landfill site, Enugu was assessed during dry (December 2015, January 2016 and February 2016) and wet (June, July and August 2016) seasons using standard procedures. Experimental soil samples were randomly collected from eighteen (18) sampling points (SS1-SS18) in each season and from six distinct sampling locations denoted as SL1, SL2, SL3, SL4, SL5 and SL6 at distances of 100m, 200m, 300m, 400m, 500m and 600m respectively away from the landfill site. SL1 represents SS1-SS3, SL2 represents SS4-SS6, SL3 represents SS7-SS9, SL4 represents SS10-SS12, SL5 represents SS13-SS15 and SL6 represents SS16-SS18. The Control soil samples SSC1 and SSC2 were collected at a distance of about 2km away from the site in dry and wet seasons respectively at a location denoted as SLC. Soil in the vicinity of the landfill site had mean temperature value ranging from 24 ± 0.46°C - 30 ± 0.36°C in wet season and 29 ± 0.6°C - 35 ± 0.03°C in dry season while pH ranged from 6.5 ± 0.05 - 7.0 ± 0.35 in wet season and 5.0 ± 0.05 - 6.9 ± 0.03 in dry season. The pathogenic bacteria revealed in this study included Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis, Bacillus sp and Clostridium sp while pathogenic fungi included Aspergillus niger, Aspergillus flavus and Aspergillus nidulans. The study showed a higher frequency of occurrence of pathogenic bacteria in wet season than in dry season while higher frequency of occurrence of pathogenic fungi were observed in dry season than in wet season. Generally, the frequency of occurrences of the isolated microbes decreased with increasing distance away from the site in both seasons and hence in the following order 100m > 200m > 300m > 400m > 500m > 600m > 2km. Statistical analysis showed that in both season the frequency of occurrences of the isolated pathogenic microbes in soil at the vicinity of dumpsite is significantly different (P < 0.05) when compared with occurrences in control soil. Therefore it is necessary for more actions to be channelled in the control of these pathogens so as to reduce potential disease outbreaks and soil borne disease occurrences. Municipal dumpsites should be located minimally at a 2km distance away from human settlement to minimize possible environmental and public health risk.
Introduction: Nontuberculous mycobacteria (NTM) are environmental opportunistic pathogens present ubiquitously. The genus Mycobacterium is included more than 188 species (<u>www.bacterio.net</u>) till 2018. The Non-tubercle mycobacterial infection occurs mainly through air, human to human transmission is not reported yet. The present study was conducted to find out the mycobacteria present in the air and soil from leprosy endemic region of India. In this study new instrument was designed to capture the air from leprosy endemic area (Purulia, WB), the instrument uses the sparger to mix air into water. The collected environmental samples such as soil and air were processed and cultured on the Lowenstein Jensen (LJ) slants. The processed sample was incubated for growth on the media. DNA was extracted from the luxuriant growth on the LJ medium. PCR was performed using universal primer 16S rRNA gene specific to mycobacterial species and species was confirmed by sequencing. The phylogenetic tree was constructed by using Multiple Alignment using Fast Fourier Transform software to establish the evolutionary association of M. leprae with NTM. Out of 6 air sample 1 mycobacterial species was isolated. 16S rRNA sequencing results revealed that it was M. simiae, which is a pathogenic bacteria and causes pulmonary infection. Similarly 32 Mycobacterial strains were isolated from 51 soil samples (62.74%), out of 32 Mycobacterial species M. fortuitum (15%) was most common mycobacterial species the other species isolated were M. parascrofulacaeum (9.37%), M. yongonense (9.37%), M. smegmatis (6.25%), M szulgai (6.25%), M. europaeum (6.25%), M. simiae (3.12%) and M. smegmatis (3.12%). The sequence of different NTMs were aligned using MAFFT software and phylogenetic tree was constructed, it was observed that the M. leprae is closely related to the M. fortuitum likewise the other species i.e. M simiae is distantly related to M. leprae. In conclusion the presence of pathogenic mycobacterial species in air and soil indicate that transmission occurs via air and soil which may cause infection in the host. That could be a reason for maintaining leprosy endemicity. This study also help to shed light on the distribution of various NTMs in environment.
Session Title: AES14 - Detection, Characterization, and Source-Tracking of Environmental Microbes: Human and Animal Pathogens in the Environment

Abstract Title:
Detection of Pathogenic Viruses and Fecal-Source Markers in Tanker Water and its Source in the Kathmandu Valley, Nepal

Primary Author Block:
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Abstract Body:
Fecal contamination of water sources is a major concern for public health. Tanker water is widely used for drinking or domestic purposes in the Kathmandu Valley. This study aimed to investigate the microbial quality and sources of fecal pollution in treated water of tanker filling stations (TFSs) and the water distributed by the water tankers (WTs). Water samples were collected from 17 TFSs in dry (n = 16) and wet seasons (n = 15) and from 30 WT samples in dry (n = 17) and wet seasons (n = 13) of 2016. Potential indicators of pathogenic viruses (pepper mild mottle virus (PMMoV) and tobacco mosaic virus (TMV)) and pathogenic viruses (human adenoviruses (HAdVs), enteroviruses (EVs), Aichi virus 1, and noroviruses of genogroups I and II (NoVs-GII)) were detected using quantitative PCR (qPCR). Bacteroidales qPCR assays (BacHum (human-specific), BacR (ruminant-specific), and Pig2Bac (pig-specific)) were used for microbial source tracking. In addition, three human viruses (HAdVs, JC, and BK polyomaviruses (JCPyVs and BKPyVs)) were tested as human fecal markers (HFMs). Escherichia coli and total coliforms were detected using Colilert reagent (Idexx Laboratories) in 52% and 87% of 31 TFS samples, respectively, and more frequently in WT samples. Of the 5 pathogenic viruses tested, EVs, NoVs-GII, and HAdVs were detected at 10, 5, and 4 TFSs, respectively. PMMoV and TMV were detected in 77% and 95% of 22 samples, respectively, which were positive for at least one pathogenic virus tested, and the positive ratio of pathogenic viruses was the highest in the group with the highest PMMoV or TMV concentrations, suggesting their use for evaluating the occurrence of pathogenic viruses in water sources. At least one of the four HFMs tested was detected in 39% of TFS samples. HFMs were detected at 2 TFSs in both seasons, while animal markers were detected at 3 TFSs of suburbs. Eight E. coli-positive TFS samples which were negative for tested markers could be due to contamination by other hosts that were untested or high limit of detections of tested assays. These findings indicated that TFSs are delivering poor quality water which needs to be improved and for proper management of the WT samples.
Abstract Title:
The Impact of Storms on Legionella pneumophila Concentrations in Cooling Tower Water
Primary Author Block:
R. L. Brigmon, C. Turick, A. Knox, C. Burckhalter; Savannah River Natl. Lab., Aiken, SC
Abstract Body:
Background: Legionellosis, or Legionnaire’s Disease (LD), is a pneumonia caused by Legionella bacteria that thrive both in man-made water distribution systems and natural surface waters including lakes, streams, and wet soil. LD is typically contracted by inhaling Legionella Disease bacteria (LDB), most often in aerosolized mists that contain the bacteria. Cooling towers have been found to be a source of Legionella in numerous LD outbreaks through aerosolization. Management of cooling towers can be a factor as the presence of stagnant water, lack of maintenance, and/or environmental conditions can cause buildup of Legionella pneumophila, the primary cause of LD. The US Occupational Safety and Health Administration (OSHA) has specific guidelines when LDB concentrations get to $10^6$-$10^7$cells/L in cooling tower water systems to prevent exposure. Methods: At the US Department of Energy’s Savannah River Site (SRS) in Aiken, SC, cooling tower water is routinely monitored for L. pneumophila concentrations (serogroups 1, 2, 4, and 6) on a monthly or quarterly basis using a Direct Fluorescent Antibody (DFA) technique. Historically, the 24 operating SRS cooling towers have had varying concentrations of Legionella in all seasons of the year with patterns that are unpredictable. The cooling towers are of varying age, water treatment system, construction, size, water supply, and geographical distribution over the 320 square miles of SRS. A stoplight system based on cooling tower water L. pneumophila concentrations has been developed to help operators control microbial growth. Results: Environmental conditions can impact L. pneumophila control in cooling towers as observed with extreme 2017 summer weather in data presented here. Cooling tower 785-A/2A concentrations went from averaging $10^5$-$10^6$cells/L to $10^7$-$10^8$cells/L after Hurricane Irma and associated extreme weather (Figure 1). Despite automated biocide addition, these increases were likely due to impact of windblown debris into the cooling tower and basin with excessive rain. Conclusions: A clean out of the cooling tower basin and repeated biocide applications were required to bring L. pneumophila down to $10^5$-$10^6$cells/L, the safe or “green” level, in this cooling tower water. Twenty-four other SRS towers were not exposed to as much debris as 785-A/2A and did not demonstrate the L. pneumophila increase despite the extra precipitation and varied from not detect (ND) range up to $10^6$cells/L as measured by DFA.
Investigation of Bacillus anthracis Outbreaks in Humans, Wildlife and Livestock in Tanzania

Primary Author Block:
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Abstract Body:
Following a period of prolonged drought, a series of suspected Bacillus anthracis (B. anthracis) outbreaks were reported between November 2016 through June of 2017 around Arusha (Monduli and Ngorongoro), West Kilimanjaro, and the Ruaha National Park in Tanzania. These outbreaks have affected hundreds of wildlife such as wildebeest, zebras, hippos, giraffe, and elephants and livestock species including cattle, sheep, and goats and resulted in considerable mortality. Over 300 human cases have also been reported, primarily consisting of skin lesions associated with contact and consumption of infected livestock and wildlife, but also several reported deaths due to anthrax. The Bushmeat Biosecurity Research Project team, together with the competent authorities from various government and non-governmental organizations, including Tanzania Wildlife Research Institute and Kilimanjaro Clinical Research Institute, has mobilized investigative teams consisting of wildlife veterinarians, microbiologists, and ecologists to the affected areas. The team observed extended congregation near watering holes because of the extended draught prevailing in the region. Blood and tissues samples collected from animal carcasses confirmed the presence of B. anthracis in smears and by real-time PCR analyses for presence of capsule and toxin genes. Further analyses have confirmed the presence of nucleic acid signatures of virulent B. anthracis spores in soil samples collected near recovery sites of dead animals. The team initiated efforts for harmonization of proactive anthrax bio-surveillance for improved prevention and control of the disease in the region together with development of timely communication strategies and implementation of animal movement controls during an outbreak. In addition to methods for ensuring quarantine of suspect animals together with vaccination of livestock within and surrounding outbreak areas, the importance of safe and effective disposal of carcasses in the field to avoid disease spread was emphasized.
Abstract Title:
Maldi-Tof Ms & Gc-Vuv As Tools for the Identification of Bacteria & their Responses to Environmental Stressors

Primary Author Block:
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Abstract Body:
The study of environmental microbial communities is of great importance as microorganisms play an essential role in biogeochemical cycles and can decompose virtually all natural compounds. Aquatic microbial communities change in response to biogeochemical alterations from anthropogenic sources. Currently, the impact of contaminants on microbial ecology is an area to be explored particularly the impact of unconventional oil and gas extraction activities (UD) on the groundwater microbiome. The use of bacterial components such as proteins and fatty acids has increasingly been applied for the taxonomic classification of microorganisms and phenotypic studies as these profiles are unique to each species and their changes are a mirror of genetic variations. Matrix-assisted laser desorption-ionization (MALDI) time-of-flight mass spectrometry (TOF-MS) allows the analysis of large molecules such as proteins with minimal fragmentation and gas chromatography (GC) vacuum ultraviolet (VUV) spectroscopy allows the analysis of volatile compounds such as fatty acids. In this work, the groundwater microbiome located near UD activities was characterized and the responses of the isolated bacteria to different chemical contaminants studied. Samples from water wells located near UD activities were collected. Groundwater samples were filtered through a 0.2 µm sterile membrane which was subsequently plated onto selective media (Nutrient agar, m-Endo Agar LES, and Aeromonas Isolation Agar). All plates were incubated at 25/37°C for 24-48 hours. Bacteria was identified using MALDI-TOF MS. E. coli, K. oxytoca, B. cereus, B. subtilis, P. aeruginosa, P. putida, P. stutzeri, and A. hydrophila were grown in Nutrient broth in the presence of 4% of ethanol, salt, toluene, propanol, and benzene. Proteins and fatty acids were extracted before analysis by MALDI-TOF MS and GC-VUV. With this work, both techniques were successfully applied for the identification of environmental microorganisms present in groundwater. These are a cheaper, simpler, and a faster alternative for the identification of environmental microorganisms. Changes in the fatty acid and protein profiles were observed after exposure to stress conditions. An increased amount of saturated and branched fatty acids was observed in the presence of the contaminants which decreases membrane permeability. The proteins present under stress conditions will be identified to determine their role in adaptation to stress. These changes can be used as environmental indicators of contamination.
Antibiotics-Based Fluorescent Probe for Selective Labeling of Gram-Negative and Gram-Positive Bacteria in Living Microbiotas

Primary Author Block:
W. Wang1, X. Chen2; 1Inst. of Molecular Med., Renji Hosp., Shanghai Jiao Tong Univ. Sch. of Med., Shanghai, China, 2Coll. of Chemistry and Molecular Engineering; Peking-Tsinghua Ctr. for Life Sci., Peking Univ., Beijing, China

Abstract Body:
Background: The differential labeling of a complex microbial sample at the Gram-stain level is of irreplaceable value to microbiologists. It is a procedure that’s practiced in nearly every aspect of microbiology, including general, medical, environmental, industrial fields, etc., however, existing labeling methods suffer from many different problems. Methods: Inspired by the vancomycin (Vanco)-based fluorescence probes that we recently reported for selective labeling of Gram-positive bacteria, we developed a polymyxin B (PxB)-based fluorescence probe to directly label Gram-negative bacteria with high specificity. The probe was prepared by reacting PxB with fluorophore NHS esters, and further isolated using HPLC. Microbiotas collected from different sources were labeled by Vanco-based and PxB-based probes directly, and analyzed by fluorescence microscopy and flow cytometry. Results: We first confirmed the labeling specificity of PxB-probe by testing against individual Gram-negative bacterial species. Then, by combining their use with Vanco-based fluorescent probes, we were able to differentially label the two bacterial groups in various living microbiotas, including mouse gut, human oral, soil, and crude oil microbiotas, with high selectivities and coverage. Both fixed and non-fixed bacterial samples were suitable. And a pilot use of the probes in labeling bacteria on heat-fixed sputum smear was also successful, suggesting its potential use in assisting traditional Gram-staining in clinics. Conclusions: The method presented here provides living-cell-compatible tools for facile differential labeling of complex bacterial samples with great potential applications in microbiology.
Abstract Title:
Use of Crassphage As A Novel Tool to Monitor Fecal Contamination

Primary Author Block:
G. W. Park, A. L. Freeland, T. F. F. Ng, J. Narayanan, A. Treffiletti, J. Vinjé; CDC, Atlanta, GA

Abstract Body:
Background: Fecal-contaminated surfaces play an important role in the transmission of human norovirus. CrAssphages are recently-discovered DNA bacteriophages that are prevalent and abundant in human feces and sewage. They are commonly found in human fecal samples, and they are increasingly studied as a potential marker of human fecal contamination of water. Methods: In this study, we tested archived human stool nucleic acid samples (n=54) that were previously tested for norovirus. In addition, nucleic acid extracts from swab samples that had been collected from 105 high touched surfaces in 21 cruise ship cabins of confirmed norovirus positive patients as well as from environmental surfaces (n=126) in public areas on the disembarkation day of the voyages. Total nucleic acids were extracted using a guanidinium-based lysis buffer and concentrated by using spin columns, followed by TaqMan-based PCR detection of norovirus and crAssphage. Results: Of the 54 stool samples, 37 (68.5%) tested positive for both norovirus and crAssphage, 8 (14.8%) positive for norovirus and 6 (11.1%) positive for crAssphage. Of the 105 swab samples collected from norovirus case cabins, 22 (21.0%) tested positive for both norovirus and crAssphage, 17 (16.2%) were positive for norovirus and 43 (41.0%) positive for crAssphage. Of the 126 swab samples from public areas, 10 (8.0%) tested positive for both norovirus and crAssphage, 11 (8.7%) tested positive for norovirus, and 56 (44.4%) tested positive for crAssphage. Conclusions: Overall, more swab samples (56.7%) tested positive for crAssphage compared to norovirus (26.0%), which suggests crAssphage may be used as a marker of fecal contamination. Additional studies, including testing animal feces, are needed to further validate the use of crAssphages to monitor human fecal contamination of environmental surfaces.
Abstract Title:
Evaluation of Homogenization Methods for Extraction of Live Bacteria and Recombinant Dna in Soil

Primary Author Block:

Abstract Body:
In many soil types, ubiquitous bacteria are present very in low quantities due to the inherent challenges of microorganism survival in environmental conditions. While, in some cases, only present in low quantity, soil microorganisms are a critical component of our ecosystem. Soil microbiome studies aim to characterize the microbial repertoire and to correlate the microbial population with soil and ecological functions. While many studies involve culturing and isolation of bacteria harvested from the soil, some organisms are hard to propagate in the laboratory or require the sample to be processed directly to maintain analyte integrity. Given time involved and complexity of culture based enrichments, many modern studies involve direct cell lysis and DNA recovery from soil followed by PCR for target enrichment and detection. In order to infer absolute taxa abundance non-conserved, targeted gene products are quantified by qPCR. While this approach is rapid and robust, qPCR is subject to inhibitory effects of soil based compounds such as humic acids and polysaccharides.In this study, we compare the recovery and qPCR detection of a modified bacterial standard from multiple types of spiked soil samples. The standard was composed of E. coli, Bacillus subtilis, and Pseudomonas fluorescens which were genetically modified with green florescent protein (GFP). The modified organism allowed the absolute subtraction of background signal originating from native organisms found in the soil samples. The soil samples were spiked with decreasing quantities of the standard. The samples were extracted through a combination of bead milling and silica spin column based DNA purification. The DNA was then quantified by qPCR against the GFP gene and the resulting data established limits of recovery for the combination of organisms. These limits were evaluated side by side with a common incubation lysis to evaluate the effects of mechanical lysis on bacterial cells in soil samples that vary in resistance to lysis. Along with the limits of recovery, cycle threshold values (ct values) were compared to evaluate the impact of PCR inhibitor release.
A Novel High-Throughput Sys. for Isolation, Cultivation and Screening of Microorganisms from Complex Ecosystems

Background: One of the key challenges in the microbiome field is to move beyond correlative gene-based profiling of bacteria to better understand their ecological function within a community. As a result, there is revitalized interest in pure culture methods, however, traditional cultivation platforms such as agar plates (AP) and liquid media tubes (LMT), are not only space and labor intensive, they are difficult to scale. Furthermore, these approaches typically favor recovery of fast growers or swarming organisms when non-selective media is used, as they can outcompete slow growers or fastidious bacteria. Therefore, in this study we utilized a newly-developed microcultivation platform, the GALT array, which consists of 50,000 miniaturized growth chambers in a 3 x 2 x 0.25 inch chip and optimized for single-cell isolation and clonal expansion. We compared this platform with AP- and LMT-based cultivation to assess maximal recovery of species from a defined and a complex community under anaerobic conditions.

Methods: The performance of the GALT array was compared with traditional culture methods using: 1) a defined 40-member bacterial consortium (comprised of slow and fast-growing anaerobes and one swarming organism), and 2) a complex community originating from human gut mucosa. All samples were seeded onto their respective cultivation platforms at ~1000 cells/ul with the same media. After 4 days, samples from each platform were assessed by 16S rRNA sequencing and compared to the starting mock community, or in the case of the complex community, the original mucosal sample. Sample preparation and incubation were performed in an anaerobic chamber.

Results: Sequencing revealed that the GALT array was as successful as LMT at recovering all the species within the mock community- even organisms at 0.01% relative abundance in the starting consortium, and had the lowest relative abundance of swarming bacteria post-incubation. The array was also as effective as LMT in recovering members from the human gut microbiota, and was especially effective in isolating members of Prevotella and Oscillospira. AP was the least effective for species recovery in both instances.

Conclusions: The GALT array is as efficient as LMT in recovering distinct species from both a defined and a heterogeneous population, while AP methods were least effective. These results demonstrate that the GALT array system is both a space-saving and effective way to profile the cultivable microbiome from various environments with broad applications in basic and applied microbiology research.
**Session Title:** AES14 - Detection, Characterization, and Source-Tracking of Environmental Microbes: Methods for Identifying and Characterizing Environmental Microbes

**Session Start Date Time:** 6/8/2018 11:00:00 AM  
**Session End Date Time:** 6/8/2018 1:00:00 PM  
**Session Primary Track:** Applied and Environmental Science

**Abstract Control Number:** 7167

**Poster Board Number:** FRIDAY - 888

**Abstract Title:**
Evaluation of Molecular Based Methods Compared to Traditional Culture Based Methods for Quantifying Ascaris in Wastewater Treatment Biosolids

**Primary Author Block:**
T. Keyzers, K. Eyre, J. Becker, E. Seagren; Michigan Technological Univ., Houghton, MI

**Abstract Body:**
Biosolids are the organic, nutrient-rich residuals resulting from wastewater treatment, and can be used for a variety of beneficial purposes (e.g., land reclamation). The EPA regulates biosolids and classifies them as Class A or Class B depending on the pathogen and indicator organism (PIO) levels. Class A biosolids contain non-detectable levels of PIOs and can be land-applied without restrictions, but the conventional means of Class A biosolids production are expensive and energy-intensive. Low-cost low-tech (LCLT) treatment methods could be an alternative for smaller wastewater treatment facilities (and sanitation in developing countries). However, equivalence of LCLT methods to EPA-approved Class A treatments must be shown and requires significant reductions of PIOs, including a 2-log reduction in viable helminth ova. Long-term storage is one potential LCLT method, which is being evaluated at the pilot-scale in this research. During the pilot-scale study, the ambient conditions, biosolids characteristics, and PIO levels were monitored. The culture-based methods for enumerating PIOs in biosolids samples are labor-intensive. Enumeration of viable helminth (Ascaris) ova in biosolids requires a sieving, floatation method followed by 28-day incubation, after which ova are observed under a microscope to enumerate and determine viability. Using this approach, we observed only a 1-log reduction in viable Ascaris ova in biosolids stored over one year, and this reduction apparently was due to increased temperatures, desiccation and/or increased volatile acid concentrations during the summer months. In contrast, a 3-log reduction in fecal coliform levels was observed within a four-month period. The use of qPCR to quantify the Ascaris internal transcribed spacer 1 region (ITS-1), which is located between the 18S and 5.8S rRNA genes, is being tested as an alternative for quantifying viable helminth ova in biosolids. Because of the nature of biosolids, several modifications to existing qPCR protocols to reduce inhibition and false positives are being evaluated, and the results of the culture-based and qPCR methods are being compared. Globally, over 800 million people are impacted by Ascaris infections. Therefore, a robust qPCR method that can be used to accurately quantify viable Ascaris ova in organic samples could be broadly used in clinical, as well as in environmental microbiology applications.
Session Title: AES14 - Detection, Characterization, and Source-Tracking of Environmental Microbes: Methods for Identifying and Characterizing Environmental Microbes
Session Start Date Time: 6/8/2018 11:00:00 AM
Session End Date Time: 6/8/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 5797
Poster Board Number: FRIDAY - 889

Abstract Title:
Recovery and Classification of 16s Rrna Genes from A Soil Metagenomic Library Using Pcr-Dependent and In Silico Approaches
Primary Author Block:
A. L. R. Santana-Pereira1, S. E. Calderòn2, D. A. Mead3, M. R. Liles1; 1Auburn Univ., Auburn, AL, 2Univ. of Puerto Rico at Humacao, Humacao, PR, 3Varigen BioSci.s Corp., Madison, WI

Abstract Body:
Background: Characterizing the extent of microbial diversity is key to understanding microbial evolution and ecology, yet estimates of bacterial diversity dependent upon PCR have inherent biases. Metagenomic sequencing has revealed the presence of the novel Candidate Phyla Radiation (CPR). Direct cloning and sequencing, while having its own inherent biases, can increase insights into the encoded genomic potential for rare and novel taxa. In this study, the phylogenetic diversity of a large-insert soil metagenomic library was compared by PCR-dependent and next-generation sequencing (NGS) approaches.

Methods: A large-insert soil metagenomic library constructed from a long-term agricultural soil (Cullars Rotation, Auburn, AL) was sequenced in column, plate and row pools using Illumina HiSeq. Reads were assembled de novo using metaSPAdes. Contigs were mined for 16S rRNA genes using BLAST against SILVA. Contigs containing hits with less than 75% homology were annotated using prodigal to predict ORFs and BLAST to compare them to the GenBank nr/nt database. The library DNA was also pooled and 16S rRNA gene amplicons were sequenced and analyzed via BLAST against SILVA. Results: A total of 295 16S rRNA amplicons were obtained that were affiliated with 10 bacterial phyla, with Acidobacteria, Actinobacteria, Bacteroidetes, Gemmatimonadetes, and Proteobacteria being the most dominant. The in silico analysis recovered 457 hits affiliated with 21 phyla, and the relative abundance was similar to that obtained by PCR. However, 14 phyla had a relative abundance lower than 1% and 9 were identified in silico only. Two large genomic clones contained 16S rRNA sequences that had a closest GenBank hit lower than 75%. Annotation of the ORFs present in these cloned genomic regions indicated functional and phylogenetic similarities to putative CPR taxa. Conclusions: In silico mining proved to be more efficient at detecting 16S rRNA candidate sequences and more sensitive in obtaining taxa present at low relative abundance. Bacterial members of the CPR phyla are unrepresented in PCR approaches due to amplification biases; in contrast, NGS coupled with bioinformatics analyses circumvented PCR limitations. Results from this study revealed a large ribotype diversity and some annotated genomic regions associated with the CPR which are not typically detected using degenerate “universal bacteria” primers to PCR amplify 16S rRNA genes. Further studies will focus on rigorous phylogenetic classification of these sequences and recovery and annotation of draft genomes.
Abstract Title: Comparison of Virus Concentration Methods for Detection of Pepper Mild Mottle Virus in River Water
Primary Author Block: E. Haramoto, T. Yamada; Univ. of Yamanashi, Kofu, Japan
Abstract Body:
This study aimed to evaluate the applicability of four different virus concentration methods to detection of pepper mild mottle virus (PMMoV), a plant virus, in river water. Small volumes of stock solutions of PMMoV (ATCC PV-227) and MS2 coliphage (ATCC 15597-B1) were inoculated into a 500-ml river water sample, which was subjected to virus concentration using a 47-mm filter to obtain a 5 or 15 ml of virus concentrate, followed by quantification of PMMoV and MS2 coliphage genomes using reverse transcription-quantitative PCR (RT-qPCR). The acid rinse method (Katayama et al. 2002. Appl. Environ. Microbiol. 68:1033-1039) and the electronegative membrane-vortex (EMV) method (Haramoto et al. 2012. J. Virol. Methods. 182:62-69) were used as representatives of methods using electronegative filters. In addition, a method using a NanoCeram electropositive filter (Argonide) was performed with two different elution buffers (Buffer A containing 1.5% beef extract, 0.05M glycine, and 0.01% Tween 80 (pH 9.0), or Buffer B containing 0.01% sodium polyphosphate, 0.001% Antifoam Y-30 Emulsion, and 0.5% Tween 80 (pH 6.7)). Among the four methods tested, the EMV method yielded the highest recovery ratios of 13.7 ± 11.6% (n = 18) and 9.5 ± 13.8% (n = 18) for PMMoV and MS2 coliphage, respectively, followed by the acid rinse method (6.0 ± 5.1% for PMMoV and 3.6 ± 4.5% for MS2 coliphage; n = 18 each), the NanoCeram electropositive filter method using Buffers A (2.6 ± 3.3% for PMMoV and 2.5 ± 2.7% for MS2 coliphage; n = 18 each) and B (1.6 ± 1.5% for PMMoV and 0.6 ± 0.8% for MS2 coliphage; n = 18 each). Based on the results of these recovery tests, the EMV method was further used to determine the occurrence of indigenous PMMoV in river water. A total of 63 river water samples were collected at 9 sites in Yamanashi Prefecture, Japan, between August and December 2016. Five liters of the water sample was subjected to the EMV method, followed by centrifugal concentration using a Centriprep YM-50 device (Merck Millipore), viral genome extraction, and (RT-)qPCR for PMMoV and other RNA/DNA viruses, such as noroviruses of genogroups I and II, human adenoviruses, and Aichi virus 1. The occurrence of PMMoV in the river water was compared with those of other viruses and conventional fecal indicator bacteria, such as Escherichia coli, to evaluate the applicability of PMMoV as indicators of viral and/or fecal contamination of river water.
The Use of Machine Learning for Identification of Microbial Biosignatures in Aquatic Communities

Microbes are ubiquitous in the world’s oceans and important players in biogeochemical cycling. Distinct environmental conditions in the oceans are known to select for different populations of microbes in specific locations. As ships transit the world’s oceans, microbes have the potential to colonize them and be carried along with them from one port to another. Here, we are seeking to characterize the ability of vessel colonization and the transport of microbes through machine learning on our vast datasets of water and surface samples of 20 ports from around the world that range in size and ship traffic. We are seeking to combine high resolution microbial community analysis using next-generation sequencing and multiple machine learning algorithm models to identify key microbial features that distinguish one location from another. Additionally, the microbial community present on both ship surface and bilge water has been characterized to detail the persistence of the geospatial biosignatures within an environment. We’ve been highly accurate in implementing our machine learning models for classifying the location of a sample solely from the microbial community composition and have discriminated vessel from water using the same tools. Our models can determine the location a sample was collected with 97% accuracy using only microbial community data. Descriptive statistics on our machine learning models have shown that we can probe datasets for insight more efficiently using members of high presence taxa, often thought to have marginal importance due to the highly abundant and ubiquitous nature of these microbes throughout aquatic environments. This work will help in understanding the potential for ships to serve as conduits for the dispersal of microbes and provide insight into what we’ve long considered a true biosignature of a particular location to be.
Abstract Title:
A New Computational Tool to Remove Contaminants from Marker-Gene and Metagenomics Data

Primary Author Block:
B. J. Callahan1, N. M. Davis2, D. M. Proctor2, S. P. Holmes2, D. A. Relman2; 1North Carolina State Univ., Raleigh, NC, 2Stanford Univ., Stanford, CA

Abstract Body:
Background: The accuracy of microbial community surveys based on marker-gene and metagenomic sequencing (MGS) suffers from the presence of contaminants — DNA sequences not truly present in the sample. Contamination in MGS experiments is common, is not fully prevented by wet lab techniques, and can lead to false conclusions in subsequent analysis. Methods: We developed the open-source decontam R package to identify and remove contaminants in MGS data. decontam uses a statistical classification procedure based on DNA quantitation data that is already collected as an intrinsic part of MGS sample preparation, and can also make use of sequenced negative control samples. Results: In a 16S rRNA gene study of 712 samples from the human oral mucosa, and 33 negative controls, the classification of contaminants by decontam was consistent with prior observations of the microbial taxa inhabiting that environment in the Human Oral Microbiome Database, and of prior observations of common contaminant genera in the scientific literature. In a test dataset of 18 marker-gene and 18 metagenomics samples from a dilution series of Salmonella bongori, the removal of contaminants identified by decontam substantially reduced batch effects associated with differences in sequencing protocols. The application of decontam to recently published data corroborated concerns about potentially spurious results caused by contamination in low biomass environments, such as the so-called placenta microbiome. Conclusion: The decontam R package is publicly available (https://benjjneb.github.io/decontam/), fully documented and integrates easily with existing MGS workflows. Our results show that decontam allows researchers to generate more accurate profiles of microbial communities at little to no additional cost.
Abstract Title:
New Luminescent Enzyme Substrates for Sensitive and Specific Microbial Detection

Primary Author Block:
J. Ihssen1, L. M. Wick1, G. Schabert1, F. Scorza2, U. Spitz1; 1Biosynth AG, Staad, Switzerland, 2Biosynth AG, Itasca, IL

Abstract Body:
Enzyme substrates have been successfully used for many years in microbiological applications in research and diagnostics. Commonly, chromogenic (e.g. indoxyl-based) or fluorogenic substrates (e.g. coumarin-based) are being used. Although luminescent substrates offer a much higher sensitivity than chromogenic and also fluorogenic substrates, they have not been used widely in microbial applications due to limited range of available enzyme-specificities and also high costs. We have synthesized a series of luminescent enzyme substrates to cover a range of important target enzymes. A first series was based on caged firefly luciferin. Free luciferin is oxidized by luciferase in a bioluminescence reaction. When the luciferin is caged by an enzyme-labile group, it is not oxidized by luciferase until the enzyme-labile group is removed by an enzyme that specifically recognizes this group. Broth containing 0.1 mM of caged luciferins were inoculated with target and non-target organisms and incubated overnight. Luminescence was measured after addition of luciferase in a microplate reader. The higher signal (RLU) of target organisms differentiated them from non-target organisms (table). Since luciferase is an integral part of these tests, we have developed an extremely stable enzyme by genetic and chemical approaches to overcome stability issues with conventional enzymes. The improved enzyme retained more than 80% of its activity after incubation for 40 minutes at 50°C, whereas a wt-luciferase showed no more activity after the same treatment. For a new class of chemiluminescent substrates that are independent of luciferase and show even higher sensitivity an initial test was done with a caprylate-caged version. The presence of Salmonella with this substrate was detected at 10^5 cfu/ml compared to the luciferin-caprylate that detected the salmonella at 10^7 cfu/ml. The experiments show that the caged-luminescent substrates lead to light production only in the presence of intended target-organisms and therefore allow a very sensitive and specific detection of microbial species.
Abstract Title:
Evaluating the rRNA/rDNA Ratio for Assessing Cell Viability Status and Implications for Microbial Source Tracking

Primary Author Block:
B. Suttner, J. K. Hatt, J. M. Brown, K. T. Konstantinidis; Georgia Inst. of Technology, Atlanta, GA

Abstract Body:
Background: Enterococcus spp. are routinely used as fecal indicator bacteria (FIB) for water quality monitoring worldwide based on the assumption that these bacteria only live commensally within human and animal gastrointestinal tracts. However, recent culture-based evidence suggests that “naturalized” populations of enterococci exist in freshwater environments with no sign of recent fecal inputs. These “environmental” strains are phenotypically indistinguishable from their enteric relatives and thus, could confound (false positive signal) water quality testing. Furthermore, enterococci are known to enter a viable but non-culturable (VBNC) state as a survival response to environmental stressors such as those found in oligotrophic aquatic habitats. Elucidating the extent to which these naturalized populations and/or VBNC state cells may confound water quality monitoring is necessary for robust public health risk assessment.

Our guiding hypothesis is that environmentally-adapted enterococci have distinct genetic and/or physiological adaptations from their enteric counterparts that underlie their differential survival in freshwater ecosystems and that this can be quantified based on gene rRNA/rDNA ratios.

Methods: We used laboratory mesocosms simulating a natural freshwater habitat, inoculated with commensal or environmental E. faecalis strains, and monitored overtime with plate counts and qPCR. Results: Our results showed that plate counts decayed over time but qPCR counts did not, invariably for both sets of strains. However, our recently developed and validated RT/qPCR assay, which uses the ratio of the 16S rRNA gene transcript (rRNA) to the gene copy number (rDNA), showed that the enteric strains experienced an increase in the rRNA/rDNA ratio by day 3 (P=0.014, paired Wilcoxian, n=9).

Conclusions: This suggests that the commensal strains might be eliciting a different stress response than the environmental strains and opens a new possibility for more reliable microbial source tracking and water quality monitoring. We will also report on our results from different growth conditions, and whole-genome transcriptomes using RNA-seq.
Abstract Title: Comparison of Next-Generation Droplet Digital Pcr with Quantitative Pcr for Enumeration of Naegleria Fowleri In Water Samples
Primary Author Block: J. Xue, S. Sherchan; Tulane Univ., New Orleans, LA
Abstract Body:
The occurrence of Naegleria fowleri in recreational waters has caught people’s attention. A rapid and accurate method to determine this pathogen in water is vital to develop effective control strategies. In this study, we compared two molecular methods: droplet digital polymerase chain reaction (ddPCR) and qPCR assays in identifying N. fowleri from clinical and environmental DNA samples. Results of clinical DNA samples show ddPCR and qPCR assays appear to have similar diagnostic specificity. A moderate agreement between ddPCR and qPCR method (κ = 0.63) over clinical DNA samples was observed. The quantity of N. fowleri targets determined in the same clinical sample by ddPCR were 1.2 to 56.5 times higher than in qPCR, which indicated potential lower sensitivity of ddPCR assay. However, our results did not show better sensitivity of ddPCR over qPCR. The N. fowleri target gene was detected in 17.7% (28/158) and 35.4% (56/158) of the water samples collected from Lake Pontchartrain by ddPCR and qPCR assays, respectively. For water samples in which N. fowleri was detected, a weak positive correlation between ddPCR and qPCR results were observed (r = 0.1), however the relationship was not significant (P-value = 0.23). The paired t-test indicated qPCR results (mean value) were statistically significantly higher than ddPCR results. The discrepancy result was probably due to the impurities in environmental water samples which may cause false amplification in qPCR. Overall, ddPCR exhibited improved precision, similar sensitivity and specificity on N. fowleri identification. To improve the reliability and performance characteristics of qPCR assay, we hereby recommend quantifying qPCR standards by ddPCR before field application. Keywords: Naegleria fowleri, Primary amoebic meningoencephalitis (PAM), ddPCR, qPCR
Abstract Title: Improved Microbial Source Tracking with Digital Droplet PCR

Primary Author Block:
J. Nshimyimana1, C. Cruz1, S. Wuertz1, J. R. Thompson2; 1Nanyang Technological Univ., Singapore, Singapore, 2Singapore-MIT Alliance for Res. and Technology, Singapore, Singapore

Abstract Body:
We addressed whether digital droplet PCR (ddPCR) could improve sensitivity and specificity of human-associated Bacteroidales genetic markers and their quantification in environmental and fecal composite samples. BacHum and B. theta, previously validated for microbial source tracking in Singapore and Southeast Asia (Nshimyimana et al, 2017), were tested in 180 samples and quantified by qPCR and ddPCR (n = 35 human stool, n = 70 domestic and wild animal feces, n = 20 sewage, n = 20 environmental and n = 35 composite samples). Quantification of BacHum by ddPCR increased specificity (from 0.63 to 0.88) and accuracy (from 0.80 to 0.93) relative to qPCR, while the B. theta marker performed similarly on both platforms (specificity = 0.98 for qPCR and ddPCR). DdPCR and qPCR quantification of environmental and fecal composite samples were highly correlated (R > 0.87, p<0.0001, n = 110) where concentrations measured by ddPCR were consistently lower than those measured by qPCR, by a factor of 2.6 ± 2.8 for B. theta and by a factor of 11.8 ± 7.8 for BacHum. When qPCR standard curves were calibrated based on ddPCR-based measurement of standards, closer agreement between qPCR and ddPCR measurements was obtained with near perfect agreement for B. theta and 2.3-fold higher qPCR values for BacHum. Thus, our work suggests ddPCR improves quantification of samples with low target concentrations by removing systematic errors associated with dilution-based qPCR standard curves. We conclude that ddPCR is a suitable tool for microbial source tracking; however, other factors such as cost-effectiveness should be considered.
Abstract Title:
Significance of Two-Step (Nested) PCR for DNA Amplification and Analysis of Biotic and Abiotic Influences on AmoA and NxrA Functional Genes in Soil

Primary Author Block:
G. Liu, T. Wu; Georgia Southern Univ., Statesboro, GA

Abstract Body:
Functional genes amoA and nxrA from the nitrification process in the soil microbial communities play important roles in regulating nitrogen cycle in the environment. Biotic factor such as tree species and abiotic factor such as underground water depth both influence the patterns of soil microbial communities. From research studies in recent years, two-step (Nested) polymerase chain reaction was suggested to be effective when targeting functional genes with low abundance in the environment. The methods of molecular biology techniques of two-step (Nested) PCR and denaturing gradient gel electrophoresis (DGGE) were performed in this research to investigate the presence of functional genes amoA and nxrA. The DNA from soil were extracted via PowerSoil DNA Isolation kit from collected study site and amplified with targeted forward and reverse primers. The amplified DNA products were then loaded into the agarose gels and gel slices contained the amoA and nxrA products were retrieved and incubated. The second step PCR was performed on the incubated amoA and nxrA products with same reverse primers and forward primers with GC clamps under the same conditions as the first step. The amplified DNA were loaded into the gel and ran in the 1X TAE buffer solution at 70 voltages for 14 hours. The statistical analyses were applied toward the results of DGGE to compare the similarities and differences of the band patterns, and cluster analyses were performed to compare the phylogenetic relationships among the soil microbial communities. After two step (Nested) PCR, bacteria functional genes amoA and nxrA were discovered from the collected soil samples at different underground water level locations (shallow and deep) with different planation growths (loblolly pine and eucalypt) for both spring and fall seasons. For the amoA gene, there were no significant pattern differences for the soil microbial communities under the influences of tree species and underground water level in spring; and no band result was identified in fall. For the nxrA gene, there were significant pattern differences for the soil microbial communities under plant species (biotic factor) in fall, and underground water level (abiotic factor) caused a significant pattern difference in spring. From this research study, the two-step (Nested) PCR was proven to be effective when studying the functional genes with low abundance in the environment, and the biotic factor (tree species) and the abiotic factor (season) influenced the patterns of soil microbial communities of the nitrification process.
Abstract Title:
Determining Lower Limits of Detection and Quantification of Quantitative Pcr Assays for Microbial Source Tracking

Primary Author Block:
J. Xue1, Y. Feng2; 1Tulane Univ., New Orleans, LA, 2Auburn Univ., Auburn, AL

Abstract Body:
Identification of fecal contamination sources in surface water has become heavily dependent on quantitative PCR (qPCR) because this technique allows for the rapid enumeration of fecal indicator bacteria as well as the detection and quantification of fecal source-associated genetic markers in the environment. Identification of contamination sources in impaired waters is a prerequisite for developing best management practices to reduce future pollution. Proper management decisions rely on the quality and interpretation of qPCR data. However, multiple methods can be used to determine the lower limits of detection (LLOD) and quantification (LLOQ) for a qPCR assay and different values are subsequently obtained for the same parameter. Additionally, low concentration responses are often interpreted differently depending on the investigators. In this study, we developed an approach to determine LLOD and LLOQ using two cattle-associated genetic markers targeting the Bacteroidales and offered interpretations that we consider appropriate. Analytical and process LLOD and LLOQ for the CowM2 and CowM3 genetic markers were determined using SYBR Green-based qPCR assays. Our results show that both analytical LLOD and LLOQ for the CowM3 marker were one order of magnitude lower than those for the CowM2 marker. The process LLOD and LLOQ for CowM3 were 20 and ten times lower than those of CowM2, respectively. These results indicate that CowM3 exhibited superior performance characteristics compared with CowM2 for fecal samples collected from our geographical area. Moreover, the method for calculating LLOD and LLOQ developed here can apply to other microbial source tracking studies.
Abstract Title:
Assessment of Qpcr Primers for Comammox Belonging to the Nitrospira Clade A Lineage
Primary Author Block:
N. Keene, D. Noguera; Univ. of Wisconsin-Madison, Madison, WI
Abstract Body:
Background: Aerating biological nutrient removal (BNR) processes accounts for nearly half of the total electricity costs at many wastewater treatment plants (WWTP). An exciting new approach to BNR involves the application of nitrification under very low dissolved oxygen (DO) concentrations (below 0.2 mg O2/L). However, it is unclear which microorganisms are responsible for nitrification in low DO conditions, with some studies unable to identify a known AOM to explain the nitrification rates observed in low DO [1]. The recent discovery of complete ammonia oxidation to nitrate (comammox) by Nitrospira (N.) in a variety of environments prompted us to investigate their presence in low DO BNR systems. We detected comammox belonging to the clade A lineage in most samples from low DO WWTP using existing polymerase chain reaction (PCR) primers; however, melting curve analysis of quantitative real-time PCR (qPCR) results indicated these primers likely overestimate comammox abundance. Therefore, we designed three new primer pairs targeting the amoA gene of existing comammox Candidatus genera to accurately detect and quantify three subpopulations belonging to N. clade A lineage. Methods: Environmental samples used in this study originated from one laboratory-scale, two pilot-scale, and one full-scale BNR systems operated with low DO conditions and from one full-scale system operated with high DO. The primer design was based on a multiple alignment of full amoA gene sequences and full particulate methane monooxygenase (pmoA) gene sequences obtained from the National Center for Biotechnology Information (NCBI) GenBank database. Results: Low efficiency and sensitivity was observed with the existing clade-level primers which was likely influenced by unspecific product formation. In addition, artifact associated fluorescence may have overestimated clade-level abundance in several samples. All qPCR assays using the newly designed primers were highly efficient, specific, and sensitive. Conclusions: Comammox amoA belonging to N. nitrosa comprised greater than 50% of the clade A population in low DO systems, suggesting that the functional relevance of these microorganisms must be considered in future low DO nitrification studies. Comammox may play an important in global nitrogen cycling, and the ability to characterize their abundance, distribution, and diversity is essential for framing their significance in both natural and engineered systems.
Abstract Title:
Novel Approaches for the Cultivation of Marine Microbes with the Metabolic Capacity for Antibacterial Activity

Primary Author Block:
S. Kennedy, C. Atkinson, R. Tawfik, B. J. Baker, L. N. Shaw; Univ. of Florida, Tampa, FL

Abstract Body:
The rise of drug-resistant microbes presents the specter of a post-antibiotic age. One fruitful area of exploration to counter this is the study of marine microbes, which are vastly understudied compared to their terrestrial counterparts. Whilst efforts have been made to understand aquatic microbial diversity, much remains to be done to modify cultivation techniques to account for the unique growth needs of marine organisms. To this end, sediment and sponge samples were used to develop cultivation modifications that facilitated the growth of a wide range of marine-derived bacteria. These included combinations of variable temperatures, 3D growth scaffolds, and incorporation of environmental extracts. Our approaches facilitated the culturing of a number of unique organisms, including a novel member of the underexplored genera Verrucosispora. To date, our methods have generated a culture collection of >200 isolates, representing a broad swath of genera from the Actinomycetales. To explore their potential as antibiotic producers, these organisms were cultured in the presence of the DNA methyltransferase inhibitor, 5-azacytidine, to epigenetically unsilence cryptic metabolic pathways. Of note, the extracts of numerous organisms produced antibacterial activity towards ESKAPE isolates, with many doing so only in the presence of the modifier. We next performed whole genome sequencing on active isolates and analyzed contigs using antiSMASH approaches, identifying clusters that display similarity to known antibiotic producing pathways. Collectively, we suggested that our novel culture methods, epigenetic manipulation and genomic approaches have the potential to identify new chemistry from understudied marine microbes that may be harnessed as future antibacterial agents.
Structure and Functional Metagenomic Evaluation of Bacteria and Archaea in Geothermal Hot Springs of Northwestern Himalayas, India

Primary Author Block:
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Abstract Body:
Comprehensive understanding of bacterial and archaeal diversity of hot water springs and its adjoining soil-mousse was undertaken using 16S rRNA. Metagenomics of new habitat facilitates identification of microorganisms possessing industrially relevant traits including synthesis of new antibiotics for tackling resistant diseases. Studied geothermal field lies in the northwestern Himalayas in India with spring’s temperature touching 96°C and soil-mousse temperature oscillating between 60 and 70°C. Samples from hot water spring were collected, shifted to laboratory on ice, genomic DNA was extracted and amplified using a two-stage PCR approach. Primers targeting the V4 variable region of bacterial and archaeal small subunit rRNA genes were used. Samples were pooled, purified and sequenced using an Illumina MiSeq Microbiology kit. Raw sequences were merged and quality trimmed to obtain a total of 56.7 Mb and 32.1 Mb of data for the samples of spring water and soil respectively. They were analyzed using MG-RAST and STAMP software. Taxonomic profiling was performed using NCBI non-redundant protein database using DIAMOND and MEGAN’s Least Common Ancestor algorithm. Functional profiling was done using SUPER-FOCUS database to estimate abundances at subsystems representing different levels of pathway specificity and for all genes in the database. The Gram-positive, endospore-forming Firmicutes were dominant in the spring water and adjoining soils followed by thermophilic Aquificae, Actinobacteria and the Deinococcus-Thermus group. Bacillus megaterium, Bacillus sporothermodurans, Hydrogenobacter sp. GV4-1, Thermus thermophiles and Thermus brockianus were the main bacterial species in the spring water. Crenarchaeota was the main archaeal phylum dominating in geothermal water and adjoining soil samples. Other predominating archaea in soil samples was Thaumarchaeota. Subsystem level diversity identified from functional metagenomics revealed dominance of genes responsible for carbohydrate metabolism, biosynthesis of cofactors, vitamins and protein metabolism. Additionally, several bacterial and archaeal sequences remained taxonomically unresolved, indicating potentially novel microorganisms in this geothermal ecosystem. Hyperthermophilic and metabolically versatile microorganisms available in such extreme environments gives us deep insight into these habitats which are further evaluated for products including antimicrobials for treatment of health issues.
Abstract Title:
Variability and Changes in Bacterial Diversity in Pristine and Disturbed Soils in An Amazonian Tropical Rainforest in Ecuador
Primary Author Block:
M. Diaz1, C. Quiroz2, P. Castillejo3, R. Simarro4, P. Jarrín5, A. Molina1; 1Universidad Central del Ecuador, Quito, Ecuador, 2Univ. Regional Amazónica IKIAM, Tena, Ecuador, 3Univ. Internacional SEK, Quito, Ecuador, 4Univ. Rey Juan Carlos, Madrid, Spain, 5Univ. Regional Amazónica Ikiam, Tena, Ecuador
Abstract Body:
Considerable areas of the Amazonian forest have been modified over thousands of years by humans into a mosaic of different habitats and soils. Most recent examples of these transformations are the colonization processes and human settlements that have been driven by the need of economic resources, such as oil extraction which has been a major development in the second half of the 20th century in Ecuador. The tropical rainforest is well known for its animal and plant diversity; however, most of its bacterial diversity remains unknown. Microbial diversity is closely related to the structure of the vegetation and the properties of soil. Thus, the community of microorganisms present at a particular site may serve as an indicator of present and past human activities. We propose that measuring microbial diversity is essential to understanding the effect of human activities on natural environments. The Limoncocha Biological Reserve (LBR) is a small remnant of tropical rainforest in the Ecuadorian Amazon region, which is surrounded by heavily modified habitats, including crops, oil extraction facilities, roads and towns. The LBR contains within its boundaries anthropogenically modified habitats. We compared the diversity and abundance of the bacterial communities in the soil present at different sites and degrees of disturbance and measured a set of nine physicochemical variables. High-throughput sequencing methods were used in the V3-V4 regions of the 16S rRNA gene to obtain a profile of the bacterial communities living at each sampled site. The information on sequence variability and diversity was combined with multivariate ecological methods (NMDS, PCA and CCA) to estimate the relationships and differences among bacterial communities in the light of physicochemical variables. We found that soil properties and bacterial communities varied significantly among sites with different degrees of human intervention. Our results suggest that the properties of soil are related to the structure of bacterial communities, but that diversity is not clearly determined by the degree of human activities.
Keywords: bacterial communities, soil diversity, bacterial ecology.
Abstract Title:
Microbial Source Tracking Reveals Fecal Contamination Occurring Within Papago Park  
Primary Author Block:
A. Burns, B. Charlton; Grand Canyon Univ., Phoenix, AZ  
Abstract Body:
Assessing water quality is important in order to ensure the safety of human health. In order to deem water quality as satisfactory, federal limits are set to meet standard thresholds. Point source and non-point source discharges introduce contaminants into a body of water causing the quality to be degraded (Seurinck, et al., 2004). The purpose of this study was to determine water quality at Papago Park through fecal indicator bacteria and microbial source tracking (MST). Papago Park is located in Phoenix, Arizona and attracts individuals for recreational purposes. The park obtains water from the Salt River Project Grand Canal where the water is pumped downstream directly from the canal, forming three man-made ponds. Through recreational activities such as swimming and fishing individuals are being directly exposed to microbial contaminants contained within the bodies of water. In order to determine water quality at Papago Park, standard collection methods were used and further analyzed for the presence of total coliforms as well as E. coli using IDEXX. The results indicated E. coli concentrations reached as high as 823.2 MPN/100mL. This concentration exceeds the standard threshold for both partial body contact at 575 MPN/100mL and full body contact at 235 MPN/100mL. However, this culture-based assessment is limited as many possible sources could be the origin of contamination. Therefore, in order to identify the specific sources of contamination, MST was used. MST identifies specific genetic marker sequences unique to a specific animal host through the use of PCR and qPCR assays. This study focused on the 16S rRNA Bacteroides; specifically, AllBac which is present in all warm blooded animals’ feces and HuBac which is only present in human feces. It was found that 50% (n=36) of the samples were positive for the HuBac sequence while 86% (n=36) were positive for the AllBac sequence. This data reveals that human fecal contamination is occurring within Papago Park. Therefore, anyone exposed to these bodies of water are at an increased risk of contracting a water borne illness. Overall, this study will allow Papago Park to improve water quality and protect public health through the identification of remediation methods.
Abstract Title:
Quantification of Anthropogenic Effects and Reliable Tracking of the Sources of Microbial Populations Along the Ganges River Using Metagenomics

Primary Author Block:
S-Y. Zhang1, D. Tsementzi1, J. K. Hatt1, A. Bivins1, N. Khelurkar1, J. Brown1, S. Tripathi2, K. T. Konstantinidis1; 1Georgia Inst. of Technology, Atlanta, GA, 2Indian Inst. of Technology, Kanpur, India

Abstract Body:
The Ganges River in India represents an ideal ecosystem to study the effects of anthropogenic inputs such as industrial and human waste resulting from intensive industrialization and urbanization on indigenous microorganisms. Herein, we applied shotgun metagenomics sequencing to study microbial community dynamics and function in planktonic samples collected along a ~700 Km river transect for two years. The transect includes urban settings (Kanpur, Allahabad and Agra cities) as well as less populated, upstream waters around Gangotri and Haridwar. Based on the likely origin of 16S rRNA and functional gene sequences recovered in the metagenomes, about 11-32% of the microbes sampled originated from soil, sludge, sewage, wastewater and human sources (allochthonous). Human gut (HG) specific sequences and antibiotic resistance genes (ARGs) were 2 to 23 times more abundant in the Ganges relative to other riverine ecosystems such as the Amazon, Kalamas (Northwest Greece) and Chattahoochee (Southeast USA) rivers, indicating intensive anthropogenic inputs. Contrary to our expectations, even the upstream samples from Gangotri and Haridwar had a similar or even higher signal of HG sequences and ARGs, some of which were also detectable downstream (> 200 Km apart). The rainy season had a major effect on the microbial community composition, which was relatively larger than the effects of geographic distance between sampling sites. Our analysis also highlighted the potential of metagenomics for source tracking purposes by following abundant individual populations from upstream samples along the river, and showing that they acquired substantial gene content differences by the time these populations were sampled downstream.
Session Title: AES14 - Detection, Characterization, and Source-Tracking of Environmental Microbes: Microbes in Soils and Water, including Markers of Fecal Contamination
Session Start Date Time: 6/8/2018 11:00:00 AM
Session End Date Time: 6/8/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 6625
Poster Board Number: FRIDAY - 905

Abstract Title:
Correlation of Fecal Source Tracking Markers with Human Health. Outcomes: Identifying Risk-Relevant Markers

Primary Author Block:
K. Zhu, B. Suttner, K. Konstantinidis, J. Brown; Georgia Inst. of Technology, Atlanta, GA

Abstract Body:
Fecal source tracking (FST) relies on host-specific markers to differentiate between human and non-human fecal contamination, ideally providing evidence of risk to public health. To this end, ideal FST markers should have signals that are reproducible across a variety of populations and that correlate with human health risk. The goal of this study is to investigate whether and how enteric infections influence the presence and magnitude of specific human-associated FST markers, using stool samples from trials where enteric infections have been characterized. We obtained human stool samples from the US, Mozambique, and Bangladesh. US samples originated from a norovirus feed study and contain healthy and symptomatic samples for each participant. We selected samples from the Mozambique and Bangladesh sets based on results from the xTAG® Gastrointestinal Pathogen Panel (GPP). To control for pathogen-dependent effects, we selected samples that were positive for norovirus GI/GII. We assembled a control group by selecting samples with no positives on the GPP and asymptomatic for diarrhea. Following DNA extraction, we performed DNA quantification using droplet digital PCR, targeting a human mitochondrial DNA (cytochrome B gene) marker and a Bacteriodes 16S rRNA (HF183/BacR287) marker, normalizing DNA counts to ng of dsDNA. The human mtDNA marker (HCytB) performed at 99% sensitivity, whereas the HF183 marker performed at 50%. In the US samples, we observed an increase in the HCytB signal from healthy to symptomatic samples (p-value = 0.06, paired Wilcoxon, n = 5, effect size = 7.8). Mozambican samples also showed an increase in the HCytB signal between control (n = 26) and symptomatic (n = 11) samples (p-value = 0.02, unpaired Wilcoxon, effect size = 0.3). HCytB results from Bangladesh samples to follow soon. This study yielded two main findings. Firstly, HF183, a high performing marker developed to detect wastewater, exhibited a decreased sensitivity when detecting individual human stools. The human mtDNA marker performed at a higher sensitivity consistently across the three populations—suggesting that human mtDNA may be a geographically stable FST marker that performs at a high sensitivity. Secondly, results from this study suggest that human mtDNA copies in stool vary with the individual’s disease state. Due to the difficulty in recruiting appropriate stool samples, the samples sizes used to investigate the mtDNA variance with disease state were small; however, further investigation with additional samples is warranted with these results.
Session Number: 82
Session Type: Poster

Session Title: AES14 - Detection, Characterization, and Source-Tracking of Environmental Microbes: Microbes in Soils and Water, including Markers of Fecal Contamination

Session Start Date Time: 6/8/2018 11:00:00 AM
Session End Date Time: 6/8/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 7003
Poster Board Number: FRIDAY - 906

Abstract Title:
Applying Microbial Community Analysis to Identify Fecal Pollution in Surface Waters and Groundwater

Primary Author Block:
L. Sassoubre, B. Durant, C. Lowry; Univ. at Buffalo, Buffalo, NY

Abstract Body:
As global populations and urbanization along coastlines rapidly grow, microbial pollution becomes an increasingly urgent problem. Microbes, specifically pathogens, are transported to the coastal environment through contaminated surface and groundwater. To protect human and ecosystem health, the sources and fate of fecal-associated microorganisms needs to be determined. Traditionally used bacterial indicators of fecal pollution can be highly variable and do not provide information about the pollution source. Next-generation sequencing (NGS) of microbial communities in coastal waters has the potential to transform the way we assess and remediate coastal fecal pollution. NGS provides a comprehensive snapshot of the microbial community composition and abundance. Previous research shows unique microbial community signatures in fecal sources and surface waters. The research presented here uses microbial community data to (1) investigate the potential of NGS as a tool to identify human fecal pollution at a Lake Erie beach frequently closed to recreational use and (2) compare the relative influence of surface and groundwater flowing into Lake Erie. Specifically, we analyzed similarities in microbial community composition in sewage influent, effluent, a Lake Erie beach, a river at the beach and groundwater flowing into the Lake. Surface and groundwater were sampled weekly over a summer to investigate temporal trends in microbial communities and potential fecal pollution events. We also analyzed microcosms of Lake Erie water seeded with 5%, 10%, 25% and 50% sewage to evaluate the detection limit for identifying human fecal pollution by NGS. We found unique microbial communities at all sites and in all microcosm samples. Results from the research will further our understanding of microbial community dynamics between surface water, groundwater and Lake Erie as well as inform the use of NGS to identify human fecal pollution.
Abstract Title:
Genetic Polymorphisms between Escherichia coli Isolates from Beach Sand and Linkage to Survival Characteristics
Primary Author Block:
K. J. Dahmer, N. Rumball, S. L. McLellan; Univ. of Wisconsin-Milwaukee, Milwaukee, WI
Abstract Body:
Escherichia coli, a fecal indicator, often exceeds acceptable levels at Great Lakes beaches, causing public health risks and economic ramifications. However, it has been proposed that E. coli forms host-independent reservoirs at Great Lakes beaches, reducing its accuracy as a fecal indicator. It has been hypothesized that E. coli is maintaining naturalized populations at the beaches of Lake Michigan, and that they pose low human health risks. It has been proposed that gaining insight into these populations’ sources and phylogeny can prevent unnecessary beach closures through proving that this E. coli is not related to fecal pollution sources that carry pathogens. We tested if there were genetic differences in E. coli isolates recovered from the berm sand of a beach compared with isolates from host sources using Clermont phylotyping and sequence analysis. Clermont phylotyping was used to classify the E. coli into phylotypes A, B1, B2, C, D, E, F or cryptic clades I-V through a quadraplex PCR, which was followed by agarose gel analysis. This determined which of the phylotype specific genes were present in each isolate. The rpoS gene from the berm E. coli isolates were also sequenced with Sanger sequencing and combined with the results of the Clermont phylotyping. This showed that B2 phylotypes were more closely related on a maximum likelihood phylogenetic tree and that the B1 phylotype was more widespread in the tree. The phylotype B2 and the cryptic clades have been suggested to encompass naturalized populations and we found B2 isolates from the sand specific isolates have polymorphisms in the rpoS gene. If E. coli survives for a long period of time in sand, this organism may not provide reliable indications of fecal contamination. Our research could lead to the utilization of different contamination indicators that would be more accurate and less costly for determining public health risk.
Abstract Title: Changes of Soil Bacterial Community Structure As A Result of Bacillus Thuringiensis Knu-07 Treatment

Primary Author Block: H. Jo, J. C. Ibal, G-S. Park, B. Jung, C. Park, Y. Jung, M-C. Kim, J-H. Shin; Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract Body: The use of plant growth-promoting bacterial agent may change the microbial community structure in soil. This study was design to observe soil microbiota changes after applying different amount of Bacillus thuringiensis KNU-07, one of the common bacterial agent used in agricultural fields in Republic of Korea. After initial treatment with different density of the agent, field soil samples were collected 2 times with 15 days term. To identify the bacterial community structure, the V4-V5 region of the bacterial 16s rRNA gene was amplified from soil metagenomes and sequenced with an Ion Torrent PGM system. The community structure and diversity were analyzed by Microbial Genomics Module in the CLC Genomics Workbench. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to predict the gene functions from soil bacterial community. To compare bacterial keystone species with or without applying the bacterial agent, Cytoscape program was applied with the Co-occurrence Network inference (CoNet). Survival rate and soil colonization of the strain KNU-07 were evaluated by using quantitative real-time polymerase chain reaction (qPCR) and direct re-isolation method. Compared to non-treated soil, dominance of class Bacilli was increased while the class Acidobacteria was decreased when the B. thuringiensis KNU-07 was applied. However, only after 15 days, the two microbial classes were converged back to type of non-treated soil. Consequently, relative amount of the strain KNU-07 was decreased after 15 days. Based on PICRUST analysis, the activity of Staphylococcus aureus was decreased and the clavulanic acid biosynthesis was promoted due to application of strain KNU-07 in soil environment. In addition, the major keystone species of bacterial agent treated soil was differ from the control soil. These results could be a basic information to extend our knowledge for applying bacterial agents in soil environment.
Abstract Title:
Outstanding Abstract Award: Spatial Impact of A Multi-Individual Grave on Soil Biochemistry and Microbial Ecology
Primary Author Block:
Abstract Body:
Decomposition of vertebrates in terrestrial ecosystems transforms the surrounding environment, altering soil physiochemistry and microbial ecology. This involves changes in carbon and nitrogen concentrations, and increased microbial and entomological activity. The majority of studies focus on surface decomposition, and effects of decomposing vertebrates in subsurface systems is not as well understood. Deeps soils tend to exhibit lower oxygen and microbial mass, which contribute to reduced rates of decomposition. This study assessed vertical and lateral changes in soil chemistry, microbial abundances and activities, and nematode ecology of a multi-individual grave four years after burial. Three deceased human subjects were interred in a grave at the University of Tennessee Anthropological Research Facility in February 2013. The grave remained undisturbed until March 2017 when remains were exhumed. Soils were collected from lateral transects radiating from the grave at two depths (0-5 cm and 30-35 cm), and within the grave at depths of 0-5 cm, 30-35 cm, 70-75 cm, and 80-85 cm. Soil samples were analyzed for physiochemical properties, including pH, electrical conductivity, gravimetric moisture, respiration rates, ammonium concentration, nitrification potential, dissolved organic carbon (DOC), and dissolved organic nitrogen (DON). The soil microbial communities were examined in terms of total bacterial and fungal abundances (via qPCR), extracellular enzyme activity, abundances of human-specific Bacteroides and nematode community profiles. Decomposition resulted in significant changes within the grave. Increased nitrification potential and nitrate concentrations were measured at 30-35 cm within the grave along with elevated bacterial and fungal DNA copy numbers suggesting a microbial bloom. The base of the grave (70-75 cm) was saturated (i.e. anaerobic), however elevated respiration rates, enrichment of bacteria-feeding nematodes and enhanced extracellular enzyme potentials demonstrated an active microbial community. In addition, human-specific Bacteroides were only found in samples from the base of the grave, where the anaerobic conditions likely allowed for persistence of these obligate anaerobes. Taken together these data show that at this site signatures of decay were still detectable within and below the buried remains four years after burial. This provides some insight into soil markers that could guide recovery, discovery, and/or age of a gravesite.
Abstract Title:
Rotenone Effects on Microbial Communities

Primary Author Block:
J. Bozzini, B. Briggs; Univ. of Alaska Anchorage, Anchorage, AK

Abstract Body:
Background: Northern Pike (Esox lucius) are classified as an invasive species in South Central Alaska. Unfortunately, pike are often illegally introduced to stocked lakes and waterways in South Central Alaska. The Alaska Department of Fish and Game has successfully treated these invasive species in Nikiski, Soldotna, and Anchorage using a chemical called rotenone. Currently, the effects of rotenone on microbial communities is not well known. This project aims to study the effects that rotenone has on microbial communities and if communities that have been exposed to rotenone are pre-conditioned to have a resistance to rotenone.

Methods: 2L water samples was collected from two lakes that were previously treated with rotenone, and two lakes that have never been treated with rotenone. Rotenone was added at 5ppb to 1L of each sample of lake water, and 1L was left untreated. After adding rotenone, the communities was incubated at 16°C (environmental temperature). Distinct samples were taken at 3 days, 1 week, 2 weeks, 1 month, and 2 months. The universal primers 515F and 806R tagged with an Illumina adapter and sample specific barcode was used to amplify the 16S rRNA gene. The PCR product from each sample was combined and sequenced using an Illumina MiSeq with the V2 300 cycle sequencing kit. QIIME was used to quality filter, de-multiplex, and assign a taxonomy (based on Greengenes) to representative 16S rRNA genes at the 97% identity level. Samples with less than 10,000 reads were removed. Results and Conclusion: The number of reads for each sample ranged between 34,000 and 87,000 with an average of 62,000 reads. 14 phyla including the candidate phyla OD1, TM6, and OP3 and 9764 operational taxonomic units (OTUs) at the 97% identity level were detected. Proteobacteria were the dominant phyla across all lakes and treatments and time points and Bacteroidetes were the second most dominant. Both Actinobacteria and Verrucomicrobia were also present, but in lower abundance. The treatments saw very little change over time at both the phyla and OTU level and there was no difference between lakes that had prior exposure to rotenone. This suggests that rotenone does not affect the microbial communities. Future studies will assess biotic degradation of rotenone compared to photo-degradation.
Abstract Title:
Structural and Functional Responses of Soil Microbial Communities to Biodegradable Plastic Film Mulching in Two Agroecosystems

Primary Author Block:
S. Bandopadhyay1, H. Sintim2, M. Flury2, J. DeBruyn1;  1Univ. of Tennessee, Knoxville, TN, 2Washington State Univ., Pullman, WA

Abstract Body:
Biodegradable plastic mulch films (BDMs) are emerging as a sustainable alternative to polyethylene (PE) based plastic films which are not biodegradable and therefore entail disposal costs and results in environmental pollution. BDMs are meant to be tilled into the soil where they are expected to biodegrade but limited research regarding the impacts of commercial BDMs on soil health has led to a reluctance in growers to adopt BDMs. We studied the effect of BDM tillage on soil microbial community structure and function over two years in two geographical locations, and compared results to the effects of PE. Identical randomized complete block designs with four replicates of seven main plot treatments were set up in two field sites: in Knoxville, TN and in Mount Vernon, WA. Treatments included four commercially available BDMs, a fully biodegradable cellulose mulch, a non-biodegradable PE mulch and bare ground. Soil samples were retrieved from 28 plots in May and September for two years in TN and WA. 16S rRNA amplicon sequencing of DNA extracted from soil was completed on an Illumina MiSeq. Data analysis was completed using Mothur® pipeline and graphics and statistics were completed in R. Community structure data was coupled with 16S and ITS qPCR data and soil functions were evaluated by conducting extracellular enzyme assays targeting α- and β-glucosidases, leucine aminopeptidase, glucosaminidase, phosphatase, and xylosidase. Soil bacterial community structure differed significantly by location (P=0.001) and season of sampling within each location (P=0.001), as determined by PERMANOVA tests. Differences in bacterial communities by treatment were not significant (P>0.05) for any season in either location. Predominant soil bacterial groups in TN were Planctomycetacia, Actinobacteria, Acidobacteria, and Alphaproteobacteria whereas in WA, Acidobacteria and Alphaproteobacteria were predominant. Extracellular enzymes assayed showed significant differences in soil enzyme activities by sampling season in both TN (P<0.001) and WA (P=0.002) as per ANOVA and Tukey HSD tests. However there were no significant differences in the functional profile of the communities between BDMs and PE mulch treatments. Limited effects of BDM tillage on soil bacterial community structure and soil enzyme activities when compared to PE suggests that BDMs are safe from a soil health standpoint. Lack of differences between plastic and bare ground treatments indicate resilience of bacterial communities to the short term application of plastic mulches.
Abstract Title: Microbial Community As Quality Indicator of Exploited Soils Consequence of Periurban Horticulture Practices


Abstract Body:
Small-scale horticulture is performed in Buenos Aires Metropolitan Area by farmers with few economical resources who have not received adequate training in agrochemicals’ use. In Moreno district, this periurban horticulture has been developed with high crop rotation and intensive use of pesticides and chemical fertilizers. In addition, oil by-products were introduced in these soils in summer 2014 by oily waste overflows of a treatment plant situated in the proximity. This scenario resulted in a decrease in crop yields that worried local producers. The aim of this work was to diagnose soil deterioration levels by a seasonal study of the microbial community composition during three years of crop production focusing on the evaluation of future restoration procedures. Samples were taken from sites with (Y6) and without (Y1) oily waste overflow history in four periods: spring 2014 (unproductive soil), fall 2015 (cabbage and green onion crop production), fall 2016 (pre-seeding soil with poultry litter amendment) and spring 2016 (post harvest soil). The oil waste treatment plant surroundings (WP) and a reference grassland (R) close to the productive plots were also sampled in the same periods. Bacterial diversity was analyzed using high throughput sequencing of the V1-V2 region of the 16S rRNA gene by Illumina Miseq and evaluating the obtained sequences in silico with QIIME pipeline. As indicators of soil nutritional conditions Proteobacteria/Acidobacteria ratios (P/A) were estimated. The impact of the 2014 overflows and a possible less extended one in 2016 was observed on WP samples, evidenced by their high P/A ratios (52 and 11.6 respectively). This effect was also detected, but deferred in time, on Y6 soils (P/A= 14.8 and 3.7 for samples obtained in fall 2015 and fall 2016 respectively). In fall 2016 Y6 P/A ratio showed a slow recovery along the time but still far from Y1 P/A values which were constant in time (ca. 1.5). On the other hand, the use of poultry litter and agrochemicals as amendments in Y1 caused an increase in copiotrophs, especially in Bacteroidetes phylum proportion and within Proteobacteria phylum composition, compared with R samples. Alphaproteobacteria abundance resulted particularly diminished with a consequent increase in Beta and Gamma Proteobacteria proportions. By checking these alterations on P/A ratio as well as copiotrophs abundances in horticultural soils, the consequences of WP overflows and the intensive use of agrochemicals and amendments were able to be detected facilitating future restoration evaluation.
Abstract Title:
Combined Effect of Abundantly Used Pesticides Chlorpyrifos and Cypermethrin on Soil Microbial Community and Associated Soil Enzymes

Primary Author Block:
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Abstract Body:
Background: In agricultural lands, soil ecosystems are continuously exposed to the agro-pollutants specifically pesticides. Recently, it has become a common practice to apply mixture of several pesticides to target multiple pests simultaneously. Soil microbial community and associated enzymes are potential bio-indicators of soil health. This study was conducted to monitor the changes in soil quality indicators as a result of contamination with chlorpyrifos and cypermethrin - two abundantly used pesticides in agriculture sector. Methodology: Mixture of chlorpyrifos and cypermethrin was applied to soil microcosms at three different application rates i.e. recommended field rate- FR, doubled the field rate- 2FR and four times the field rate- 4FR. To examine changes in soil biochemical properties, eight soil enzymes (catalase, dehydrogenase, β-glucosidase, invertase, acidic phosphatase, alkaline phosphatase, protease and urease) were monitored during the experiment. For analyzing changes in soil microbial community dynamics, culture dependent biodiversity indices like CD (Colony Development) index and EP (Ecological Physiology) index were used. At the end of experiment, setup most affected by pesticides' exposure was subjected to 16S rRNA Amplicon Pyrosequencing to probe culture independent community shift. Results: Chlorpyrifos and Cypermethrin altered the soil microbial population, depicted by strong changes in the culture based biodiversity indices i.e. CD index and EP index. Soil enzymatic studies revealed significant changes in acidic/alkaline phosphatase, dehydrogenase, β-glucosidase and urease. Pyrosequencing data projected noticeable community shift in the soil microbial community exposed to the pesticides in comparison to the control. Chao1 and Shannon diversity indices depicted decrease in richness and diversity of bacterial community on pesticides exposure. Pesticides caused decrease in the relative abundance of some environmentally important bacterial genera such as: Acinetobacter, Agrobacterium, Bacillus, Caulobacter, Mesorhizobium, Pseudoxanthomonas and Rhizobium. While relative abundance of certain genera like Acidobacterium, Candidatus, Gemmatimonas, Kaistobacter, Leptothrix and Pseudomonas increased in response to pesticides’ exposure. Conclusions: By monitoring bio-indicators for soil health, the hazardous nature of Chlorpyrifos and Cypermethrin was affirmed which consequently leads to the disturbed soil ecosystems.
Effect of Suppressive Soils in Goldenberry (Physalis Peruviana) and Fusarium Oxysporum

Primary Author Block:
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Abstract Body:
Background: The rhizosphere microbiome has a large effect on plants, impacting various physiological attributes, such as tolerance to biotic stress caused by soil borne pathogens. The composition of this microbiome is determined by the microbial community present in the soil and the interaction between these microbes and the roots. Some soils have been known to hold microbial communities that suppress disease development (suppressive soils). Several regions in Nariño (Colombia) have historically shown lower incidence of vascular wilt produced by Fusarium oxysporum (Fox) in Physalis peruviana crops. The aim of the present study was to evaluate the biological activity of soils with potential suppressiveness from Nariño to control vascular wilt in Physalis peruviana. Methods: The rhizospheric soil from farmlands with two different management systems, one conventional (Puerres) and the other organic (Gualmatan) were collected. Three treatments were evaluated (e.i., conducive soil+Fox, Gualmatan+Fox, Puerres+Fox) and compared with the corresponding controls. For each treatment, the same soil with a history of disease development (conducive soil) was inoculated with the suppressive soils (10% w/w). P. peruviana plants were transplanted, and grown for 71 days. Results: Disease parameter, incidence and severity were quantified. The results indicated that plants grown in conducive soils inoculated with the pathogen showed a 47.5% and 47.7% of incidence and severity of vascular wilt, respectively. In contrast, soils inoculated with the organic potentially suppressive soils showed significantly smaller values. For the treatment Gualmatan+Fox,7.4% of incidence and 8.7% of severity. For the treatment Puerres+Fox there was a 6.5 % severity and 6.7% an incidence. To determine the microbial community present in the rhizosphere and surrounding soils form the suppressive soils, treatments, and the original conducive soil, molecular analysis were conducted sequencing 16Sr RNA and ITS amplicons. Conclusions: Preliminary results showed that the rhizosphere of suppressive soils have a high abundance of bacterial taxa related to the Actinobacteriales and myxococcales order, species associated with this taxonomic groups have shown antagonism against plant pathogens. In conclusion, our experiments showed that soils from Nariño hold microbial communities with suppressive potential against the development F. oxysporum, and that communities may have a better potential for the control of devastating pathogens.
Abstract Title: Microbial Response to Alternative Waste Stream Fertilizers in the Root-Zone of High Value High-Density Apple Orchards

Primary Author Block:
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Abstract Body:
Background: Soil microbial communities play a major role in determining success of high value crops such as apple. A growing concern shared by apple growers in the mid-Atlantic is the maintenance of a profitable but also sustainable management system. While the use of synthetic fertilizers has a positive effect on apple yield, it is detrimental to water and waterways (e.g. Chesapeake Bay). One potential key to improving soil, plant health, and positive plant-microbial feedbacks is the use of management practices that are associated with root-zone symbiotic microbes. Recent research has shown that some soil amendments alter orchard rhizosphere microbiomes, leading improved apple yields by conferring resistance to pathogens. The aim of this research is to survey the effects of alternative waste stream fertilizers on the rhizosphere of apple orchards in the mid-Atlantic region. The long-term research goal is to assess if these waste streams support positive plant-microbial interactions relative to synthetic fertilizers.

Methods: Apple trees were planted at three sites in north Virginia, and northern Maryland. Two mulch treatments (mulch and no-mulch) and four fertilizer amendments (none, compost, compost with calcium nitrate, calcium nitrate) were applied to each site. Bacterial DNA was isolated from root-zone soil at all three sites (n=96) while fungal DNA was isolated from just two sites (n=64). The bacterial 16S rRNA gene and fungal ITS region were amplified, and sequenced (Illumina MiSeq platform).

Results: Visualization of the beta diversity, UniFrac, and Bray-Curtis distances have shown that the bacterial and fungal communities were strongly influenced, as expected by the orchard’s location. Multivariate tests using adonis and anosim, as expected revealed that mulch but not compost caused shifts in bacterial communities, and to a lesser extent fungal communities, compared to synthetic fertilizer.

Conclusions: These first year experiment’s results point to the importance of organic amendments on microbial communities. It is expected that analysis of the root-zone communities after 2 to 3 years after amendment will show more shifts in microbial communities, and greater indications of mycorrhizal fungi development. It is also notable that trends suggest alternative waste organics support greater apple stem growth, and thus may have other beneficial effects that also support their use in place of less sustainable synthetic fertilizers.
Characterizing the Effect of Different Organic Cover Crop Techniques on Microbial Diversity

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Background and Methods: The microbial community within a piece soil is essential to the fertility of soil and success of agricultural crops, yet a substantial portion of microbial life within the soil remains uncultivated and under-explored. Many species of bacteria can play mutualistic as well as harmful roles in the life cycle of a crop, and specific practices can play a deciding factor in generating different microbial communities. One such strategy, cover cropping, involves planting a specific specie(s) of plant and then mowing down to the ground (without harvesting) for restoring and recycling nutrients in the soil and developing beneficial biota. To understand how various cover cropping techniques affect soil microbial communities, we used next-generation sequencing to compare the bacterial presence under a single-, multi-species, as well as a lack of cover crop influence. An experimental plot was established with the three different cover crop treatments in triplicate. After six weeks of cover crop treatments, a broccoli crop was planted. Soil samples were taken at key points throughout the experiment. From each soil sample, specific nutrient levels were determined. Additionally, DNA was isolated and Illumina sequencing was performed using universal primers specific for the V3 and V4 regions of the 16s rDNA gene. Sequence analysis is ongoing, but we are comparing the presence of known mutualistic and parasitic microbial genera. Results and Conclusions: Data thus far indicate that the cover crop techniques correlate with differential impact on soil fertility and crop-outcome. An overall 6-7% increase in active carbon content in single- and mega-mix treatments, respectively, as well as an overall 10% decrease in the control group were observed. On average, plants from the control group exhibited the greatest mass but also the least length while the mega-mix group demonstrated the lowest mass but the greatest length. The mega-mix group demonstrated a 6% increase in average leaf area while the single-mix exhibited a 6% decrease when compared to the control group. As the experiment is on-going, the microbial data, carbon and nitrogen content measurements, and final broccoli assessment are yet to be interpreted. These results will be the first to systematically investigate how cover crops alter microbial communities of the soil. While our study is specific to an agricultural setting, our results will contribute to the growing body of knowledge of how specific plant communities can change the microbial ecology of the soil.
Abstract Title:
Effects of Tillage on Soil Microbial Communities in A Soybean Cropping System

Primary Author Block:
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Abstract Body:
Tillage is a common practice employed in agriculture, used to raise soil beds for seed planting, disrupt weeds, and allow tracks for water to flow during irrigation. However, the repeated disruption of soil structure each year can increase erosion and leaching of nutrients from soil, and have deleterious effects on soil health. As a result, no-till management has been employed as a way to mitigate these negative effects. The current study was conducted to examine the long-term effects of tillage on the size, activity, and composition of microbial communities in soybean (Glycine max L.) field plots maintained under till and no-till conditions for 14 years. Surface soil (0-5 cm) was collected from 12 replicate plots from each treatment in the fall of 2014 after soybean harvest and analyzed for microbial biomass, enzyme activities, and bacterial community composition. Microbial biomass carbon was significantly higher in no-till compared to tilled plots (p=0.0053). Soil enzymes activities linked to phosphate mineralization (phosphatase), organic matter turnover (β-glucosidase), and general microbial hydrolytic activity (FDA hydrolysis) were up to 1.8-fold higher in no-till plots (p<0.05). Analysis of 16S rRNA gene sequences indicates that diversity (Chao1, ACE, and Shannon indexes) was 5-8% greater in tilled plots compared to no-till (p<0.05). At the phylum level, Proteobacteria were the most abundant in both tilled and no-till plots, making up approximately 31% of the bacterial sequences in both treatments. At the subphylum level, Betaproteobacteria were higher in no-till (p=0.0082) while Alphaproteobacteria more abundant in tilled plots (p=0.0351). Bacterial taxa known to contain plant-growth promoting bacteria were also higher in tilled plots, including Rhizobiales (p=0.0327), and Paenibacillaceae (p=0.0037), although these groups make up a relatively small proportion of the total community, from 0.16 (Paenibacillaceae) to 4% (Rhizobiales) of bacteria in tilled plots. Given the small magnitude of the differences in microbial community composition between treatments, differences in soil activities were attributed to the increase in microbial biomass. These results demonstrate how tillage practices can have a great impact on the size and activity of the microbial community in agricultural soils while having only minor effects on community composition.
Loss of Microbial Diversity Exacerbates Spread of Antibiotic Resistance in Soil

Primary Author Block:
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Abstract Body:
Loss of biodiversity is a major threat to the ecosystem processes upon which society depends. Natural ecosystems differ in their resistance to invasion by alien species, and this resistance can depend on the diversity in the system. Little is known, however, about the barriers that microbial diversity provides against microbial invasion. The increasing prevalence of antibiotic-resistant bacteria is a serious threat to public health in the 21st century. Here we explored the consequences of reduction in soil microbial diversity for the dissemination and implantation of antibiotic resistance. We investigated the relationship between soil microbial diversity and the invasion of antibiotic resistance using a dilution-to-extinction approach coupled with high-capacity quantitative PCR. Microbial diversity was negatively correlated with the abundance of antibiotic resistance genes, and this correlation was maintained after accounting for other potential drivers such as incubation time and microbial abundance. Our results demonstrate that high microbial diversity can act as a biological barrier against the spread of antibiotic resistance. These results fill a critical gap in our understanding of the role of soil microbial diversity in the health of ecosystems.
Transmission and Source-Tracking of Environmental Campylobacter jejuni Isolates

Primary Author Block:
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Abstract Body:
Campylobacter is the leading cause of bacterially-derived gastroenteritis worldwide and has a significant impact on human health. Campylobacter resides commensally in poultry and other livestock, but results in severe gastroenteritis when ingested by humans, occasionally leading to post-infectious disorders, including Guillain-Barre syndrome and reactive arthritis. Currently, it is unknown which of these sources of Campylobacter are responsible for the majority of human infections. Due to the increasing rates of antibiotic resistance in Campylobacter, it is important to identify the cause of Campylobacter infections. Recently, Whole-Genome Sequencing (WGS) has been employed to aid in the tracking of gastrointestinal pathogens back to a source. As this approach has been successful for other organisms such as E. coli and S. typhimurium, it may be useful in analyzing Campylobacter. Due to the high mutability of Campylobacter, we first determined the efficacy of this approach at identifying and tracking environmental isolates of Campylobacter. To do this, we randomly selected two Campylobacter chicken isolates from a poultry farm in East Tennessee and subjected them to WGS. Following sequencing, we used these strains in the laboratory to recapitulate the Campylobacter infectious cycle, going from chicken, to poultry meat, to murine model of infection, with samples at each step being subjected to WGS. This novel approach provided insights into whether WGS as a means of source tracking is a valid option for human Campylobacter infections. Subsequently, we isolated Campylobacter from agricultural, food, and human clinical samples in East Tennessee and sequenced these genomes. Using insights from our transmission study, we analyzed these genomes using a bioinformatics pipeline developed at Oak Ridge National Laboratory and determined whether the source of human infections in East Tennessee could be identified.
Abstract Title:
Occurrence of Listeria Monocytogenes in Milk and Milk Products in Ethiopia
Primary Author Block:
Abstract Body:
Background: Listeria monocytogenes is of major significance in human and veterinary medicine. Most human Listeria infections are foodborne and the association of contaminated milk and dairy produce consumption with human listeriosis is noteworthy. In Ethiopia, there is limited data regarding the prevalence of L. monocytogenes in raw bovine milk and dairy products. The aim of this study was, therefore, to determine the prevalence of L. monocytogenes in raw bovine milk and dairy produce.
Methods: A total of 443 raw milk and milk product samples (raw milk: n=343; pasteurized milk: n=65; Yogurt: n=20; cheese: n=15) were microbiologically analyzed following methods recommended by the U.S. Food and Drug Administration Bacteriological Analytical Manual to isolate Listeria spp. Results: The overall prevalence of Listeria spp. was 28.4% and specifically that of L. monocytogenes was 5.6%. Taking the prevalence of Listeria spp. into consideration, cheese was found to be highly contaminated at 60%, followed by pasteurized milk samples (40%), raw milk (18.9%) and yogurt (5%). Considering the prevalence of Listeria monocytogenes only, raw milk had the lowest contamination (2.04%) while cheese (26.7%) had the highest, followed by pasteurized milk (20%) and yogurt (5%). Conclusions: Raw milk and milk products produced in urban and peri-urban areas of central Ethiopia were contaminated with pathogenic bacteria, L. monocytogenes. The detection of this pathogen in raw milk and milk products warrants an urgent regulatory mechanism to be put in place and also the potential role of milk processing plants in the contamination of dairy products should be investigated.
Abstract Title:
Prevalence of Mycotoxins in Selected Nigerian Fermented Foods

Primary Author Block:
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Abstract Body:

Background: In Africa, fermented foods and beverages play a significant role in contributing to food security and their production is dominated by informal processing sectors that make use of different traditional processing methods which affects the quality and safety of the final products. This study evaluated the safety quality in terms of mycotoxin prevalence of some fermented foods obtained from Nigerian markets. Methods: Fermented food samples (n = 191) including maize gruel (ogi), sorghum gruel (ogi baba), melon seed (ogiri), locust bean (iru) and African oil bean seed (ugba) from Southwest Nigeria were quantified for 23 mycotoxins, including aflatoxin B1 (AFB1), fumonisin B1 (FB1), and sterigmatocystin (STE) using liquid chromatography-tandem mass spectrometry. Results: Data obtained revealed that 82% of the samples had mycotoxins occurring singly or in combination. FB1 was present in 83% of ogi baba samples, whereas 20% of ugba samples contained AFB1 (range: 3 to 36 µg/kg) and STE was present in 29% of the ogi samples. Ochratoxin A was found in ogi baba, iru, and ugba, at mean levels of 6, 6, and 9 µg/kg, respectively, whereas, roquefortine C was only detected in iru and at a low incidence rate (range: 10-14 µg/kg). HT-2 was the most frequently occurring trichothecenes in the samples and the level of deoxynivalenol in all the positive samples (n = 18) reached a maximum of 118 µg/kg. In terms of multi-mycotoxin contamination, FB1 + FB2 + FB3 + STE + AFB1 + alternariol + HT-2 co-occurred within one sample. Conclusions: This is the first study to report a wide range of previously unreported mycotoxins in iru, ogiri and ogi consumed in Nigeria. The extent to which the analysed mycotoxins contaminated these food commodities justifies the need to enact fungal and mycotoxin mitigation strategies along the food chain.
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Session Title: AES17 - Foodborne Pathogens: Detection, Surveillance and Tracking
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Session Primary Track: Applied and Environmental Science
Abstract Control Number: 5900
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Abstract Title:
Global Hlth. Risks Associated with Bushmeat Consumption in Tanzania

Primary Author Block:

Abstract Body:
Background: Bushmeat, meat from wildlife species, is a source of animal protein that is harvested (mostly illegally) in many parts of Africa, including Tanzania, but also is often smuggled into the US and Western Europe. Given the documented evidence of the presence of dangerous zoonotic pathogens amongst wildlife harvested for bushmeat in Tanzania, our study was designed to assess the biological risk and potential for impact on human health from bushmeat consumption. Methods: A comprehensive stratified random sampling approach was used to map the prevalence and the distribution of anthrax, Brucella and Coxiella during rainy and dry seasons in villages and surrounding markets in three targeted ecosystems (Serengeti, Ruaha, and Selous). Microbiome sequencing analyses of the V3-V4 region of the 16S rRNA gene was performed on a subset of 56 fresh and processed bushmeat samples from species including wildebeest, buffalo, eland, giraffe, and zebra, recovered from 21 villages in and around the Serengeti ecosystem, and provide further evidence for the presence of nucleic acid signatures of genera representing these three select pathogens as well as other dangerous pathogens. Results: Preliminary analysis of real-time PCR results from more than 3000 samples collected from over 100 different villages across the three ecosystems identified signatures of Bacillus anthracis (0.41%), Brucella (1.04%), and Coxiella (0.32%) in bushmeat harvested and sold in this region. The microbiome analysis on the samples indicated that there are no significant differences in the phyla diversity between the wildlife species and sample conditions (fresh vs processed). Furthermore, the 2-Dimensional Principal Coordinates Analysis indicates that the wildlife species tend to cluster based on regions, seasons, and sample conditions. Conclusions: Taken together, the results of our investigations provide evidence of the presence of DNA signatures of especially dangerous zoonotic pathogens in bushmeat sold or prepared for consumption in Tanzania. In the long-term, our research will provide a rational basis for defining and mitigating the public health risks associated with the harvesting, trade, and consumption of bushmeat.
Abstract Title:
Effect of Abattoir Processing on Microbial Quality of Cattle Carcasses At A Municipal Abattoir in Ibadan, Nigeria

Primary Author Block:
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Abstract Body:
Background: Unhygienic slaughtering and processing of food animals in Nigerian abattoirs resulting in contamination of meat is a major food safety challenge. This study assessed the effect of abattoir processing on microbial quality of cattle carcasses at a municipal abattoir in Ibadan, Nigeria. Methods: Four hundred swab samples of cattle carcasses (200 Before Washing - BW and 200 After Washing - AW) were taken from 50 randomly selected cattle slaughtered and dressed at Bodija municipal abattoir, Ibadan. Four swabs samples from brisket, thigh, neck and forelimbs muscles per carcass BW after flaying, and four AW with water were pooled as one each, leading to 50 BW and 50 AW pooled samples. Swab samples of butchers tables (n=17), slaughter-hall floor (n=17), wall (n=17), and water used in washing carcasses from the primary source (n=3) and secondary sources (n=3) were collected. Samples were cultured using standard bacteriological procedures to determine Aerobic bacteria Plate Count (APC), Enterobacteriaceae count (ENT), Salmonella, Escherichia coli and Listeria monocytogenes contamination of samples. Data were analysed using descriptive statistics, t-test and ANOVA at p<0.05. Results: The mean APC (LogCFU/cm² ± SD) of BW was 8.96±2.34 and AW was 8.99±2.37. The ENT count (LogCFU/cm² ±SD) was 7.87± 2.67 and 8.11± 1.90 for BW and AW, respectively. The carcass BW and AW mean counts (LogCFU/cm² ±SD) for Salmonella were 8.40±.40 and 8.49±.58, E. coli 7.67 ± 2.55 and 8.19 ±1.28 and L. monocytogenes 8.37±.47 and 8.49±.58, respectively. Salmonella counts after washing carcasses were significantly higher than before washing (p<0.05). Tables, floors and walls including water had bacterial counts that exceeded international standard. Tables had the highest ENT counts (8.54±0.32LogCFU/cm²) while wall had lowest (8.32±0.33). The mean Salmonella count (LogCFU/mL) of water from the secondary source (9.093±0.015) was higher than from primary source (8.615±0.045). Conclusions: The bacterial loads of carcasses, equipment and water were high. Implementation of Hazard Analysis Critical Control Points, maintenance of hygienic abattoir environment and meat processing as well as use potable water and clean equipment are recommended to reduce the risk of meatborne infections.
Abstract Title:
Aflatoxins Investigation & Mycobiota of Selected Marketed Smoked Dried Fish Samples in Ado Ekiti, Ekiti State, Nigeria

Primary Author Block:
E. D. Fagbohun1, O. U. Lawal2; 1Ekiti State Univ., Ado Ekiti, Nigeria, 2Evangel Univ., Abakaliki, Nigeria

Abstract Body:
The mycobiota and aflatoxins contamination in selected smoked-dried fish sold at Oja Oba market in Ado-Ekiti, Nigeria was investigated. Samples of smoked dried fish (Bonga, Cat, Wet African Shad, Butter and Sole) were randomly sampled and purchased from five different marketing sites located at Oja Oba main market in Ado-Ekiti. Fifty samples, ten from each related species were analysed. Mycological analysis was done with Potato Dextrose agar using direct plating, washing and dilution methods while the fungi were identified using standard procedures. The moisture content of the fish samples were determined by oven drying at 105°C for 4½ h. The aflatoxin extraction, quantitative and qualitative determination was carried out and the results were analysed using Duncan multiple range test. Eleven different fungal species belonging to six genera were isolated from the smoked dried fish samples from the market sites. The fungal species were Aspergillus niger, A. fumigatus, A. tereus, A flavus, Absidia sp., Rhizopus sp., Penicillium sp., Penicillium citrinum, Penicillium italicum, Mucor sp and Fusarium moniliformis. Aspergillus flavus and Penicillium sp. had the highest rate of occurrence among the fungi isolated. Aflatoxin B1 and G1 was found in Cat fish (Gynmallabes typhus), West African Shad (Ilisha africana) Sole fish (Cynoglossus brown) while it was not detected in the rest. The aflatoxin B1 and G1 concentration ranged from 2.731 to 4.031 µg.kg⁻¹ and 2.015 to 3.528 µg.kg⁻¹ respectively while the fungal count ranged from 4.7x10² to 9.1x10⁴cfu.g⁻¹. The moisture content ranged from 21.1 to 28.8%. This study showed that smoked fish displayed for sale at different market sites in Oja Oba market in Ado-Ekiti, Nigeria were contaminated with species of fungi and aflatoxin which pose a great threat on the health of the consumers. Fish samples should be well smoked and dried to reduce moisture content, and the samples for sale should be kept in a covered container or show glass to reduce settling of droplets and spores.
Abstract Title:
Microbial and Aflatoxin Content Analyses of Plantain Chips Sold in Open Markets in Benin City Metropolis, Nigeria

Primary Author Block:
S. A. Igbinedion, S. E. Omonigho, F. O. Okosun; Univ. of Benin, Benin City, Benin City, Nigeria

Abstract Body:
Background: Plantain is a major staple food in most parts of humid tropical Africa. Plantain chip is a ready-to-eat food because it is prepared and/or sold by vendors and handlers for immediate or later consumption without further processing or preparation. This study was aimed at assessing the microbiological, physicochemical quality and aflatoxin content of plantain chips sold in open markets in Benin City, Edo State. Methods: Plantain chips samples (10 samples from each market) were purchased from three open markets: Oba, Santana and Uselu markets in Benin City. The culture media (nutrient and potato dextrose agars) were prepared according to manufacturer’s instruction. Nutrient agar was used for the isolation of bacteria while potato dextrose agar, containing antibacterial agent, was used for fungal isolation. The plates were incubated at 28±2oC for 48 hr for bacterial and 28±2oC for 5 to 7 days for fungal enumerations, respectively. Developed colonies were isolated and purified by repeated sub-culturing and stored on agar slants as stock cultures. Characterisation and identification of isolates was carried out using colonial, morphological and biochemical characteristics. Physicochemical analyses including pH, titratable acidity and moisture content were also carried out. The pH value was determined using an electrode pH meter (JENWAY 3020) while moisture content was determined by oven drying method. Titratable acidity was determined by titrating 0.1N sodium hydroxide against 10 ml of supernatant of homogenised sample, using phenolphthalein indicator. Results: The total bacterial counts ranged from 2.10±0.80 x 10⁵ cfu/g (Santana market) to 8.80±0.21 x 10⁵ cfu/g (Uselu market) while the total fungal counts ranged from 6.00±2.55 x 10² cfu/g (Santana market) to 6.00±2.00 x 10³ cfu/g (Uselu market). Bacterial genera isolated include; Staphylococcus aureus, Streptococcus sp., Escherichia coli, Bacillus subtilis and Micrococcus spp. while five fungal genera; Aspergillus niger, Aspergillus flavus, Penicillium, Trichoderma, Mucor and Saccharomyces spp. were isolated. The pH values ranged from 5.40±0.07 (Santana market) to 5.60±0.10 (Uselu market), while moisture content ranged from 8.90±1.30% (Oba market) to 15.00±1.22% (Santana market). Titratable acidity ranged from 0.19±0.03 g/l (Uselu market) to 0.27±0.04 g/l (Santana market). Conclusion: Proper handling during processing and packaging is the best approach for reducing microbial contamination with routine monitoring of foods sold in open markets by health agencies.
Abstract Title:
Salmonella Infection among Apparently Healthy Food Handlers in Convenient Food Productions
Primary Author Block:
D. Mohsen1, M. Abbas2;  1Animal Hlth.Res. Inst., Cairo, Egypt, 2Suez Canal Univ., Ismailia, Egypt
Abstract Body:
Background: Food contamination may occur during its production, processing and preparation. The risk of food getting contaminated depends largely on the health status of the food workers. unsafe food handling and processing can assist as a potential for the transmission of a diversity of disease causing agents. Hands of ready-to-eat food service workers have been shown to be routes in the spread of foodborne disease, mainly because of poor personal hygiene. Our objective was to evaluate the efficacy of hand washing practices and sanitation before commencing work among food handlers in the convenient food productions.
Methods: A total of 250 hand samples and Stool samples were collected respectively. involving 100% of the same food handlers, From 5 selected convenient food outlets preparing ready-to-eat foods. The workers’ cleaned and disinfected hands. Sampled examined for Total Plate Count (TPC) of Salmonella. Bacteria were isolated and counted using standard methods. Stool Samples collected examined for pathogens contamination, Biochemical and sensitivity tests were done using Kirby-Baur disk diffusion technique to identify the species of pathogens.
Results: The hand samples revealed highest bacterial count 7.2 x 103 cfu.cm. The results shown that all the examined sample exceeded the legal limit for food surfaces or hands of < 100 cfu.cm when the average bacterial counts on hands were compared. Sixty percent of the TPC analyzed exceeded the legal limit and only 18% of the food handlers had no bacteria detectable on their hands. Hand washing practice after toilet and touching food with bare hands were independent predictors of infectious enteric diseases among the food handlers.
Conclusion: The present study revealed high prevalence of Salmonella contaminations among participant’s food handler, hand hygiene is unsatisfactory and may have serious implications for public health due to contamination of food from handlers’ hands. Therefore, Workers pre placement in service, further training and food hygiene should be provided to all handlers with regular inspection to improve adherence of good hand washing practices and food safety practices.
Abstract Title:
Occurrence, Genetic Diversity, Typing of Enterotoxin and Biofilm Genes, and Antibiogram Patterns of Staphylococcus aureus Isolated from Bovine Raw Milk Samples from the Indian State of Rajasthan

Primary Author Block:
D. K. Dahiya, S. Sharma, V. Sharma; Post Graduate Inst. of Vet. Ed. and Res. (Rajasthan Univ. of Vet. and Animal Sci. at Bikaner), Jaipur, India

Abstract Body:
Background: Staphylococcus aureus is the main pathogen found inflicted in dairy animals and humans. Strategies to prevent its infections could be better framed by deciphering their genotype and other pathogenic traits. Here, we conducted the study for the first time from the Indian state of Rajasthan having biggest inventory of milch cattle and the main contributor of milk in the country. Methods: From 13 major cities of the state during 2014-2015, 368 bovine raw milk samples were collected. Microbiological and PCR based methods were used to enumerate S. aureus. Restriction fragment length polymorphism (RFLP) within a coagulase gene (coa) was used to explore their genetic diversity. PCR based methods were adopted to type their enterotoxigenic and biofilm-producing genes. Next, MIC strips were used to evaluate their antibiotic resistance patterns. Chromogenic agar and PCR was used to further ascertain the methicillin-resistant S. aureus (MRSA). Results: In all, 73 S. aureus isolates were retrieved and only 30 strains were found positive for coa with 09 distinct patterns ranging from 730 to 1130 bp. Dendrogram analysis categorized the strains into 15 different genotypes whereof, cluster I, IV, V, and VI were the most prevailing ones. Only following enterotoxigenic genes, i.e., sec, sea, and seb were found positive in the proportion of 30%, 10% and 3.3% respectively, among isolates. All isolates harbored the biofilm producing genes icaAD and eno, whereas the frequency of other genes bbp, fib, bap, cna, sasC, fnbB, sasG, ebpS, clfB, and fnbA was in the order of 6.6, 10, 27, 53, 77, 80, 80, 90, 93 and 97%. The majority (27/30, 90%) of the strains exhibited resistant to multiple antibiotics and 15 of them were found resistant to methicillin i.e MRSA positive. All 15 MRSA strains possess the mecA gene. However, all strains were susceptible to kanamycin and might be utilized for prophylaxis of S. aureus originated cattle infections in the region. Conclusions: We found that bovine raw milk samples of Rajasthan were contaminated with the multidrug resistant, biofilm producing, enterotoxigenic strains of S. aureus and their accidental consumption may pose serious infection to humans because of its high zoonotic potential.
Abstract Title:
Detection of Drug Resistant Pathogens and Toxic Adulterants in Fresh and Tetra Pack Milk Samples in Karachi, Pakistan

Primary Author Block:
J. Samad1, S. Sikandar1, S. U. Kazmi2, H. Qureshi1; 1Habib Univ., Karachi, Pakistan, 2Dada Bhoy Inst. of Higher Ed., Karachi, Pakistan

Abstract Body:
Background: Milk and dairy products are a potential source of food borne infections worldwide. The developing countries, including Pakistan, have been at higher health risk due to poor food quality and adulteration. This study was conducted to assess the microbial quality and chemical hazards of fresh and tetra pack milk. Methods: Samples (n=35; 25 raw/fresh milk and 10 tetra pack) were collected in sterile containers from average to above-average socioeconomic areas of Karachi. Samples were directly inoculated on selective and differential media for 24 hours. Results were finalized by growth, morphology and biochemical tests. Antibiotic susceptibility was checked using Kirby Bauer test. Common milk adulterants like formalin, soap, starch, sucrose, benzoic acid and water content were also checked using standard methods. Results: The raw milk was stored at cold temperature but salesmen were not using gloves while dispensing. Raw milk samples were contaminated with human pathogens including E.coli (36%), Enterobacter aerogenes (52%), Proteus vulgaris (48%), Shigella (28%), S.epidermidis (56%), Citrobacter (16%), Klebsiella (20%), S.aureus (100%), Salmonella spp. (4%), GPC(Enterococcus/Micrococcus) 88% and Bacillus spp.(32%).Tetra pack pasteurized milk were free from microbes, only one contained Shigella. Antibiotic resistance pattern showed resistance to Amoxicillin (74%), Augmentin (49%), Ceftazidine (46%), Ceftriaxone (41%), Ciprofloxacin (9%) and Gentamicin (4%). Adulterants were detected in both tetra pack and fresh milk samples. All the samples had slightly acidic pH & water content ranging from one tenth to three tenth, only 37% samples did not have additional water. Starch and soap were absent. Sucrose was present in 10% of the samples, benzoic acid in 43% and formalin in 74%. Conclusions: The possible causes of microbial contamination can be handling, storage, transportation and milk drawing methods. Good hygiene and handling practice should be followed to minimize it. Pasteurization is required to preserve the milk however strict policies and monitoring are needed to maintain quality standards. Exposure of microbes and harmful adulterants in both, fresh and tetra pack milk, should be regulated to prevent health risk.
Detection and Molecular Characterization of Staphylococci from Eggs of Domesticated Chickens

Abstract Body:
Background: Eggs of domesticated hens are considered as more nutritious and healthier than eggs of farmed chickens. Previously, many studies have reported the presence of pathogenic bacteria inside eggs. Nonetheless, these studies were conducted on eggs of farmed chickens. No study has been carried out on isolation and characterization of bacteria from eggs of domesticated chickens, that are free living in the natural habitat, fed on diverse types of foods and have longer life as compared to farmed chickens. The aim of the present study was to isolate and characterize strains of staphylococci from eggs of domesticated chickens. Methods: Eggs (n=275) of domesticated hens were collected from different villages of Khyber Pakhtunkhwa province, Pakistan, from November 2016 to March 2017. Presence of staphylococci was determined by inoculating the egg content on a selective medium i.e. mannitol salt agar. Initial identification of staphylococci was made on the basis of microbiological assays such as microscopy, biochemical tests, and growth characteristics. Genus specific primers for staphylococci, species specific primers for Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) were used for PCR. Further identification to species level was made using Vitek 2 system. Antibiotic susceptibility was performed using disc diffusion agar assay. Genetic fingerprinting of Staphylococcus xylosus strains was carried out using Pulsed field gel electrophoresis (PFGE). Results: Sixty two eggs were positive for staphylococci identified as S. xylosus, S. lentus, S. sciuri, S. haemolyticus, S. gallinarium and S. aureus. The predominant isolated species was S. xylosus, which was isolated from 26 eggs. PFGE patterns show heterogeneity. S. aureus were isolated from two eggs and only one was identified as MRSA. Conclusions: To the best of our knowledge, this was the first study on the detection and characterization of bacteria from eggs of domesticated hens. In contrast to our previous study in which a high number S. aureus strains were isolated that showed high level of antibiotic resistance, most of the strains isolated from the brown eggs were non-pathogenic species. Further comparative studies are suggested.
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Session Start Date Time: 6/8/2018 11:00:00 AM
Session End Date Time: 6/8/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 7039
Poster Board Number: FRIDAY - 931

Abstract Title:
Presence of Toxoplasma gondii DNA in Food and Water Od Sch. Restaurants in Armenia, Quindio, Colombia

Primary Author Block:
O. A. Zamora Velez, F. M. Lora Suarez, J. E. Gómez Marin; GEPAMOL, Quindio Univ., Armenia, Colombia

Abstract Body:
Background: Toxoplasma gondii is an important foodborne parasite. It can be transmitted via encysted bradyzoites in meat and by oocysts contaminating vegetables or water, where the children are one of the most affected populations. In present work our aim was to evaluate the overall exposure of food, water and surface to DNA of T. gondii at three school restaurants randomly selected in the city of Armenia, Quindio (Colombia), by using conventional PCR. Methods: We collected and analyzed samples of food (meat and vegetables), water (from faucet and boiled water ready to drink or use for preparing food) and living (hand surface of operators) and inert (kitchen bench) surfaces. Meat samples were cut into 5 g pieces. The vegetables were treated with glycine or wash solution (Tween 0.1% and sulfamic acid 1%) and mixed through stomacher. The DNA from the samples was extracted by using Qiagen and Wizard kit. Results: The T. gondii DNA was detected by using conventional nested PCR for B1 sequence. We found by using conventional PCR: 1 of 6 (16.6%) samples positive in meat; 1 of 10 positive samples (10%) in living surfaces; 1 of 10 samples (10%) positive at inert surfaces; 1 of 9 samples (11.11%) positive in boiled water and 1 of 3 positive (33.3%) in water samples taken at faucet. Conclusions: The use of the conventional PCR for B1 sequence could be a good way to detect DNA from T. gondii. This is the first study in Colombia that report T. gondii DNA in food and water samples in school restaurants and the first in evaluating this parasite in vegetables and living and inert surfaces samples. The findings indicates that there exist many potential sources for Toxoplasma infection in food and water in school restaurants in Colombia. Funded by Colciencias Grant Number: 111372553376.
Abstract Title:
Microbiological Safety Indicators of Canastra Cheese in Brazil
Primary Author Block:
G. Z. Campos1, U. M. Pinto1, C. Hoffmann1, B. D. G. M. Franco1, M. Landgraf1, G. A. Lacorte2, L. A. Cruvinel2; 1Univ. of São Paulo, São Paulo, Brazil, 2Federal Inst. of Minas Gerais, Bambuí, Brazil
Abstract Body:
Canastra Artisanal Minas Cheese is made from raw milk manufactured by rural producers of the Serra da Canastra region, in Brazil. The production process employs fermentation driven by an endogenous culture called "pingo", originated from the whey which is collected from the cheese made in the previous day. The use of raw milk is a risk factor for food safety. Therefore, it is important that foodborne pathogens are controlled after the minimum ripening period of 22 days required by legislation. This work aims to determine the microbiological safety indicators of the Canastra Cheese from 70 rural properties in the region of Canastra. To date, cheeses from 47 properties have been analyzed. Total coliform counts, Escherichia coli and Staphylococcus coagulase positive were performed on Petrifilm® paltes (3M). The detection of Salmonella spp. was carried out by ISO 6579:2002 method and Listeria monocytogenes according to ISO 11290-1:1996/(A)1:2004. The Enterobacteriaceae count was determined by the APHA method 9.62:2015. The pH analyzes were performed according to IAL 463-IV and the Water Activity in an Aqua Lab analyzer. The results of the microbiological analyzes were compared with the requirements of the legislation for this product (Table 1). Table 1: Compliance and non-compliance of Canastra Artisanal Minas Cheese samples with legislation, and values of pH and water activity, with standard deviation. 

<table>
<thead>
<tr>
<th></th>
<th>General</th>
<th>Total Coliform</th>
<th>E. coli</th>
<th>Staphylococcus coagulase positive</th>
<th>Salmonella spp.</th>
<th>pH</th>
<th>Water Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comply</td>
<td>23</td>
<td>36</td>
<td>35</td>
<td>32</td>
<td>47</td>
<td>46</td>
<td>5,47(0,24)</td>
</tr>
<tr>
<td>Do not comply</td>
<td>24</td>
<td>11</td>
<td>12</td>
<td>15</td>
<td>0</td>
<td>47</td>
<td>5,59(0,34)</td>
</tr>
</tbody>
</table>

The results show that 24 of the 47 samples analyzed were unsatisfactory according to at least one requirement of the legislation. There are no reference values for
pH and Aw values in legislation for this product. However, we stratified these data in Table 1 according to the results of the microbiological analyzes. The results suggest that there is no significant difference (p <0.05) in pH and Aw values between the two groups. Our data show that no sample was contaminated with Salmonella and L. monocytogenes was found in only one sample analyzed so far. However, high counts of E. coli or total coliforms or Staphylococcus coagulase positive were observed in some samples. This results indicate a great need to implement and improve hygienic-sanitary conditions in the production of these cheeses due to the high numbers of samples that did not comply with the legislation.
Real Time Multiplex-QPCR for the Simultaneous Detection of Foodborne Pathogens from Environmental Swabs

Primary Author Block:
A. N. Queen1, K. Hirneisen1, V. Sathyamoorthy2, A. Datta2, D. Williams-Hill1; 1U.S. Food and Drug Admin., Irvine, CA, 2U.S. Food and Drug Admin., Laurel, MD

Abstract Body:
Background: Escherichia coli O157:H7 (EHEC), Salmonella spp.(Sal), and Listeria monocytogenes (Lm) are the most significant pathogens with respect to FDA regulated food products. Standard methods for environmental swab sample analysis target only one pathogen in a sample and require preparation of multiple pre-enrichment media per the Bacteriological Analytical Manual; this is time consuming (demanding on average > 5 days of analysis), and cost/labor intensive. Current FDA research aims to identify a universal enrichment broth for simultaneous multiple pathogen detection via real-time multiplex qPCR (mqPCR). Testing and validating a rapid and accurate method to simultaneously enrich and detect multiple targets would provide a critical reporting tool. The study objectives are two-fold: (1) Simultaneous enrichment of EHEC, Sal, and Lm from environmental swab samples for identification through mqPCR, and (2) Performing a single lab validation of these methods. Methods: Plastic, a common food preparation surface was selected for swabbing. A plastic tub was sanitized and outlined with large (4x4 inch) and small (1x1 inch) grids for swabbing per AOAC guidelines. Duplicate grids were spiked with 100 μl of high (HS) or low (LS) Brain-Heart Infusion (BHI) broth culture concentrations of EHEC (1.82x10⁸ CFU/ml; 1.82x10⁵ CFU/ml), Sal (1.62x10⁸ CFU/ml; 1.62x10⁵ CFU/ml), and Lm (2.66x10⁸ CFU/ml; 2.66x10⁵ CFU/ml). Large and small surface area swabbing was performed using Dey-Engley broth moistened sponge-tipped swabs and cotton-tipped swabs respectively followed by a two-hour room temperature incubation. Swabs were enriched for twenty-four-hours in Buffered Listeria Enrichment Broth (BLEB) broth at 35°C followed by DNA extraction, and mqPCR. Results: mqPCR analysis detected multiple microorganisms in the HS grids in both the large and small surface areas. Lm and EHEC were detected with Ct values of 25.74±1.09 and 31.79±1.58, respectively, in BLEB broth enrichment from HS large grid sampling (n=2). There was a significant difference for Lm recovery/detection in HS of large vs. small surface areas (p<0.02), despite equal amounts of spiked organisms. Conclusions: Simultaneous enrichment of multiple bacterial foodborne pathogens would enhance assessment of processing facility sanitation while using fewer resources and reducing reporting time to 24-48 hours. This method would serve as an asset between laboratories and compliance across the food science landscape.
Abstract Title:
Real Time Bacterial Colony Growth Detection on An Agar Plate Using Laser Speckle Decorrelation

Primary Author Block:
S. Han, J. Park, K. Lee, Y. Park; KAIST, Daejeon, Korea, Republic of

Abstract Body:
Background: Culturing bacteria on an agar plate is a standard method to test its response to various conditions. In the process, inoculating the bacteria is a time-consuming step that takes 12-48 hours. To shorten the time needed for inoculation, we propose a laser speckle decorrelation method to detect the real-time response of bacterial colony growth on an agar plate. Methods: A laser beam impinges on a bacteria spread agar plate through a turbid plate, which generates a random laser pattern called ‘speckle.’ A digital camera records the speckle illuminated agar plate for 25 minutes. The recording is repeated every thirty minutes until the colony formation is completed. We calculated the autocorrelation of the time-lapse speckle images with the time parameter value $\tau = 15$min. Results: The correlation value from an agar plate without bacteria reaches 0.9 within two hours and remains at the high correlation level after that. The high correlation after two hours demonstrates that there is no manifest movement on the agar plate. However, the agar plate with bacteria shows a severe correlation decrease between 6-12 hours. This is due to the colonial growth movement of the bacteria. After 12 hours, the correlation graph increases again representing the steady phase after the full growth of the colony. The correlation graph from a sample with a lower bacteria concentration level shows a similar shape as the bacterial result before, but the correlation decrease came out a few hours later. This is because the lower bacteria concentration needs a longer time to grow up to a certain level for the detection. The antibiotic agar plate with bacteria gives a similar result as the agar plate without bacteria because of no bacterial activity. Conclusions: This method tracks the real time growth of the bacteria colony that enables early stage detection of bacterial response to a given environment before fully inoculated. This shortens the time needed for bacterial response detection such as antibiotic susceptibility test. For further application, the examination of bacterial activity inside food is possible with the same methodology. However, the mechanical instability of food such as chicken breast tissue should be overcome.
Abstract Title:
Beta-Hemolytic Bacteria Selectively Trigger Liposome Lysis, Enabling Rapid and Accurate Pathogen Detection

Primary Author Block:
R. Sum1, M. Swaminathan1, S. K. Rastogi1, O. Piloto2, I. Cheong1; 1Temasek Life Sci. Lab., Singapore, Singapore, 2Protean Labs LLC, Medley, FL

Abstract Body:
Background: For more than a century, blood agar has been the only test for beta-hemolysis. Although blood agar cultures are highly predictive for bacterial pathogens, they are too slow to yield actionable information. Other molecular and immunological assays have been developed, however they require a priori information of the pathogen and often require enrichment to achieve sufficient sensitivity.

Methods: In our study, we replaced blood agar with liposomes encapsulating either Hoechst 33342 (L-Hoechst) or sulforhodamine B (L-SRB). We tested these fluorescent liposomes against a panel of 5 beta-hemolytic bacteria (Bacillus cereus, Streptococcus pyogenes, Staphylococcus aureus, Clostridium perfringens, Listeria monocytogenes), 2 alpha-hemolytic bacteria (Streptococcus pneumoniae, Streptococcus salivarius) and 4 gamma-hemolytic bacteria (Streptococcus oralis, Staphylococcus epidermidis, Lactococcus lactis, Escherichia coli). Results: Beta-hemolytic pathogens are able to lyse L-Hoechst whereas alpha and gamma-hemolytic bacteria have no effect. By analyzing fluorescence kinetics, beta-hemolytic colonies cultured on agar could be distinguished in real time with 100% accuracy within 6 hours. In addition, end point analysis based on fluorescence intensity and machine-extracted textural features could discriminate between beta-hemolytic and co-cultured control colonies with 99% accuracy. In broth cultures with L-SRB, high loads of beta hemolytic bacteria were detectable in several hours while alpha and gamma-hemolytic bacteria remained negative even the next day.

Conclusions: This strategy, which we termed “Beta-hEmolysis Triggered-release Assay” (BETA) has the potential to enable the same-day detection of beta-hemolysis with single-cell sensitivity and high accuracy. There are many ways in which BETA could be customized for particular applications, such as the use of hemolysin-specific phospholipids and selective growth media.
Evaluation of Different Enrichment Protocols and Testing Algorithms for the Detection and Isolation of Salmonella Species from Food Samples During Two Recent Outbreak Investigations

Primary Author Block:

Abstract Body:
Background: Non-typhoidal Salmonella is the most common cause of bacterial-related foodborne illness in the United States. Public health laboratories play an important role in foodborne outbreak investigations. With the advent of new technologies including whole-genome sequencing (WGS), there will be an increase in the number of Salmonella outbreaks that would have been undetected previously. Two recent Salmonella outbreaks presented the opportunity to evaluate the effectiveness of our current enrichment methods and testing algorithms.

Materials/Methods: Universal Pre-Enrichment broth (UPRE), Buffered Peptone Water (BPW), and Lactose Broth (LACB), were evaluated as primary Salmonella enrichment broths for 12 different food products. The enrichment broths were incubated for different time periods at 37°C. Following the primary enrichment, aliquots were transferred to RVR Broth and incubated for 24 hours at 42°C. RVR broths were washed, pelleted, and incubated at 95°C for 20 minutes. An in-house developed real-time PCR was used to screen enrichment broths for the presence of Salmonella DNA. DNA-positive enrichment broths were then plated onto selective agar plates and incubated for 24 hours at 37°C. Salmonella-like colonies were isolated and screened by MALDI-TOF mass spectrometry, then confirmed identification with conventional biochemicals. In addition, these isolated colonies were analyzed by PFGE and WGS. Results: Salmonella DNA was detected in 2 out of the 12 food products tested. PCR results from enrichment broths indicate that both UPRE and BPW promoted more optimal Salmonella growth as compared to LACB. Salmonella-like colonies were isolated from both broths and were rapidly identified as Salmonella species. Analysis by PFGE and WGS determined relatedness between one food product isolate and an outbreak A and excluded the other food product isolate as a possible source of outbreak B. Conclusion: It is essential to reevaluate existing testing methods and algorithms as technology changes. The advent of new technologies such as MALDI-TOF MS and WGS allowed these outbreaks to be investigated using the most streamlined, efficient methodologies available. Ongoing studies will allow us to optimize the enrichment protocols used in the laboratory to screen food products quickly and accurately during an outbreak investigation.
Abstract Title:
Antibiotic Resistance Profiles of Escherichia coli and Enterococci Isolated from Retail Meat in Connecticut

Primary Author Block:
C. R. Nishimura1, A. J. Pellissery1, M. Surendran Nair2, P. G. Vinayamohan1, K. Venkitanarayanan1; 1Univ. of Connecticut, Storrs, CT, 2Univ. of Minnesota, Saint Paul, MN

Abstract Body:
Antimicrobial resistance (AR) has emerged as a serious public health threat. The Centers for Disease Control and Prevention reports that AR infections account for two million illnesses and 23,000 deaths annually in the US, with over $20 billion as direct health-care costs and $35 billion in lost productivity. The National Antimicrobial Resistance Monitoring System (NARMS) was established as a surveillance system to track changes in antimicrobial susceptibility of enteric bacteria found in ill people, retail meats, and food animals. With animals being recognized as a reservoir of AR bacteria, animal-derived foods represent a major source of these bacteria to humans. Thus, there is a need to determine the prevalence of AR bacteria in animal-derived foods, and delineate their AR profile for devising appropriate control strategies. Escherichia coli and enterococci have emerged as important nosocomial pathogens in humans, with food suggested as a potential source. Although studies have indicated the prevalence of AR E. coli and enterococci in a variety of meats, no reports are available in the northeast. This study screened 202 samples of retail meats, including 80 chicken, 51 ground turkey, 36 ground beef, and 35 pork chops procured between 2016 and 2017 from commercial markets in three Connecticut counties for enterococci and E. coli. NARMS procedures were followed for the isolation and biochemical identification of the isolates, which were further confirmed by Matrix Assisted Laser Desorption/Ionization technology. Enterococci was isolated from 151 (74%) retail meat samples, with increased prevalence in ground meats (95%). E. faecalis (82%) was the most commonly isolated enterococci, followed by E. faecium (11%). E. coli was isolated from 58 (28%) of the retail meats, with greater isolation from retail poultry (53%). Over half of the E. coli were resistant to tetracycline, and one-third were resistant to aminoglycosides and penicillin. Antibiotic-resistant E. coli was most frequently isolated from ground turkey (67%) compared to other meats. Nearly 95% of enterococci isolates were resistant to at least one antibiotic and 55% were multi-drug resistant (resistant to 3 or more drugs). These isolates were resistant to lincosamide (92%), streptogramin (84%), tetracycline (58%), aminoglycosides (18%), quinolones (1.3%), and vancomycin (<1%). Results indicate a high prevalence of AR enterococci and E. coli in retail meats sold in Connecticut, and justify continued surveillance to monitor AR bacteria in meats from other counties in the state.
Abstract Title:
Longitudinal Environmental Sampling of Small Organic Farms for Foodborne Pathogens and Indicator Bacteria As Part of the Harvest Initiative
Primary Author Block:
K. Cook1, A. Oladeinde1, J. Plumblee Lawrence1, G. Zock2, C. Hall1, L. Wiggins1, S. House1, E. Line1, K. Hiett3, A. Zimeri2; 1USDA, Athens, GA, 2Univ. of Georgia, Athens, GA, 3FDA, Laurel, MD
Abstract Body:
The HARVEST Initiative (Healthy Affordable Renewable Variety: Enabling Sustainable Trade) seeks to analyze farming techniques and address food security in Northeast Georgia and beyond. The popularity of farmers’ markets has increased in recent years because of an increased interest in local foods and organic produce. As part of this project, we conducted a longitudinal study to address the microbiological quality of samples taken from four organic farms using biological soil amendments of animal origin (BSAAO) for growing produce. We monitored for the abundance of indicator bacteria (E. coli, coliform and Enterococci) and pathogens (Salmonella and Campylobacter) in BSAAO, soil, irrigation water (IW), and surface water (SW) samples collected bi-monthly during the produce growing season. In addition, squash samples were collected at farmers’ market at harvest. Campylobacter was not detected in any sample. Salmonella was detected in only one BSAAO sample by culture, however, 32.0 %, 30.8 % and 0 % of BSAAO, soil and produce samples, respectively, were positive for Salmonella by molecular analysis. As part of analysis for indicators, we found that the concentration of E. coli was below our limit of quantification in all samples. The concentration (standard error) of coliforms in BSAAO, soil, IW and SW was 4.95 ±0.26, 6.20±0.094, 1.85±0.22 and 1.05±0.076 log colony forming unit (CFU) g-1 or 100mL-1, respectively. For enterococci the average concentration was 4.94±0.35, 4.49±0.33, 1.00±0.0077, and 2.43±0.13 log CFU g-1 or 100 mL-1, respectively. The concentration of coliforms and enterococci on squash from the farmers’ market was 7.08±0.15 and 6.23±0.13 log CFU 100 g-1. Collectively, our results demonstrate the need for caution when selecting methodologies and indicators used to confirm the microbiological quality of organic farm samples. BSAAO may also be an important reservoir of indicator bacteria. Further research is needed to identify specific farming practices that enhance produce safety taken from organic farms using BSAAO.
Abstract:

Background: Campylobacter is a major foodborne pathogen in humans and also the predominant cause of infectious abortion in sheep in the U.S. Despite the fact that ruminant animals are increasingly recognized as important reservoirs for Campylobacter, limited information is available about the molecular epidemiology and antimicrobial resistance profiles of sheep Campylobacter. Methods: To close this knowledge gap, here we conducted two separate studies to determine the distribution and molecular characteristics of Campylobacter from sheep. Each of the studies involved 80 commercial sheep, 40 of which were medicated with tetracycline in feed, while the other 40 received no antibiotics. Fecal samples (once a week) and bile samples (at necropsy) were collected for the isolation of Campylobacter. The bacterial isolates were identified by MALDI-TOF and then analyzed by antimicrobial susceptibility tests. Additionally, PFGE and MLST analysis were performed with some selected isolates.

Results: The results revealed that 87.0% and 61.3% of the fecal and bile samples were positive for Campylobacter (C. jejuni and C. coli). Regardless of the medication, all tested fecal isolates apart from one C. coli were resistant to tetracycline, the only antibiotic approved for the prevention and control of sheep abortion in the U.S. Notably, ciprofloxacin resistance was detected in 6.4% and 95.0% of the C. jejuni and C. coli isolates, respectively, revealing a drastic difference in the ciprofloxin-resistance rates between C. jejuni and C. coli. There was no significant difference (P > 0.05) in antibiotic resistance profiles between the tetracycline-mediated group and the non-mediated group. PFGE and MLST analysis of randomly selected C. coli isolates revealed that nearly all of them shared identical PFGE patterns and belonged to a single ST type, ST902. Additionally, all of the analyzed ciprofloxacin-resistant C. coli isolates carried the Thr-86-Ile mutation in GyrA.

Conclusions: It can be concluded that 1) tetracycline-resistant Campylobacter is highly prevalent in commercial sheep operations, regardless of the medication with tetracycline; 2) because of the high prevalence of resistance, tetracycline is no longer useful for the control of Campylobacter-induced sheep abortion in the U.S.; and 3) a fluoroquinolone-resistant C. coli genotype (ST902) has become the predominant strain in sheep. These findings provide new insights into the molecular epidemiology of Campylobacter in sheep and useful information for the control of sheep abortion.
Abstract Title:
Longitudinal Field Study Evaluating the Spillover of Antibiotic Resistant Escherichia coli from Poultry to Humans in Ecuador

Primary Author Block:
H. Hedman1, J. Eisenberg1, G. Trueba2, D. Vinueza Rivera2, R. Zurita Herrera2, J. Villacis Barrazueta2, L. Zhang3, S. Meda4, G. Gavilanes5, E. Krawczyk1; 1Univ. of Michigan, Ann Arbor, MI, 2Univ. San Francisco de Quito, Quito, Ecuador, 3Michigan State Univ., East Lansing, MI, 4Michigan State Univ., East Lansing, MI, 5Univ. San Francisco de Quito, East Quito, Ecuador

Abstract Body:
Small-scale farming operations in rural communities often prescribe high amounts of antibiotics for industrial meat production breeds of chickens (e.g. broilers). In contrast, free-ranging local varieties of backyard chickens receive almost no antibiotics. Recent evidence suggests that backyard chickens in proximity to broiler chickens have increased levels of phenotype and genotype antibiotic resistance. We conducted a seven-month longitudinal study aimed to examine whether backyard chickens and children serve as sentinels for detecting antibiotic resistance spread into the environment from broiler chickens in northwestern Ecuador. Escherichia coli isolates were identified from children (n = 1144), backyard chickens (n = 1323), and 1-day-old broiler chickens purchased from vendor sources (n = 253). Isolates were examined for their resistance phenotypes to 12 antibiotics and selected resistance genes. Phenotype resistance profiles fluctuated over time for human and backyard chicken samples. In contrast, broiler chicken resistance profiles remained high for all antibiotics tested. We also detected that households closest to households raising broiler chickens yielded significantly greater phenotype resistance levels among avian and human samples (general additive model; p < 0.005). The same blaCTX-M gene was detected in both human and chickens. These results likely suggest that small-scale broiler farming operations may function as sources of environmental antimicrobial exposure for the surrounding human and animal populations. Our results indicate that industrial meat-producing animals may introduce antibiotic resistance into other animal breeds, likely through horizontal gene transfer spillover events into backyard breeds and humans.
Abstract Title:
Prevalence of Shigatoxin-Producing Escherichia coli in Different Types of Flour

Primary Author Block:
S. Schlager, C. Schlagenhauen, S. Neubauer, K. Hauser, E. Edler, B. Springer, F. Allerberger; Austrian Agency for Health and Food Safety (AGES), Graz, Austria

Abstract Body:
Background: Two recent outbreaks of Shigatoxin-producing Escherichia (E.) coli (STEC) infections, one centered in the United States and one in Canada, were caused by contaminated flour [1,2]. We studied the prevalence of STEC in flour from Austrian retailers. Methods: Thirty-one different types of flour (22 produced from wheat, five from rye and four from spelt) were obtained in five Austrian groceries in July 2017. They originated from seven different mills and were tested for the presence of STEC using ISO/TS 13136:2012. In short, organisms were cultured in buffered pepton water for 24 h and PCR was used to test for stx1 and stx2. In case of positivity viability of STEC was tested on SMAC and TBX agar. For the confirmation of STEC in human samples primers and probes published by Reischl et al. (J Clin Microbiol. 2002; 40:2555-65) were used. Shigatoxin (stx) subtyping was performed in accordance with the PCR scheme of Scheutz et al. (J Clin Microbiol. 2012; 50:2951-63). Results: Six out of 31 flour samples (19.4%) were stx-positive after enrichment (3x wheat, 2x rye and 1x spelt). In five of these six samples we detected stx2g and in one wheat flour sample stx2b. Viable STEC were detectable in four of the six positive samples. While we failed to isolate purified STEC in three specimens, one of the four culture-positive samples yielded a strain of STEC O36:Hrough. In contrast, none of 212 STEC-positive human fecal samples (collected from July to December 2017) available to the Austrian Reference Center for E. coli yielded detectable stx2g; 37 of these 212 fecal samples tested positive for stx2b (17.5%).

Conclusions: Both stx2b and stx2g are genes considered to exhibit only low virulence. We were unable to identify the presence of high virulence stx2a, stx 2c or stx 2d in flour, and so far no Austrian cases of STEC infections have been epidemiologically linked to flour or consumption of raw dough. Our findings indicate that flour may pose a risk for STEC infections also in Austria.
Session Number: 112
Session Type: Poster Talk
Session Title: Let's Get Rid of It! Bioremediation of Toxic Compounds
Session Start Date Time: 6/8/2018 12:15:00 PM
Session End Date Time: 6/8/2018 1:15:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 8704
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Jon Weeks; FDA, Laurel, MD
Abstract Body:
Abstract Title:
Comparison of Polystyrene and Polyethylene Degradation by Insect Larvae
Primary Author Block:
A. Navlekar, D. Carr; Texas Tech Univ., Lubbock, TX
Abstract Body:
In recent years, microbial plastic degradation has become a huge area of interest. Currently, plastic disposal occurs by recycling, conversion to a different product or chemical degradation. Until very recently, polyethylene (PE) required certain chemical additives to make it degradable. To find a more prudent way to deal with this challenge, researchers have concentrated their efforts on using bioremediation as a solution to the plastic disposal problem. Yang et al. (2014) showed that polyethylene was degraded by the gut microbial community of Plodia interpunctella (larvae of Indianmeal moths). While the gut microbiota of these larvae may possess plastic degrading capabilities, the process itself is unclear. The types of microorganisms involved and any changes in their abundances during PE degradation is still unknown. We have recently completed the metagenomic study of polystyrene (PS) degradation by Tenebrio molitor (larvae of the Mealworm beetle). We showed that the native gut microbial community of Tenebrio itself can degrade polystyrene without much change to its community composition or structure. However, there were certain changes seen in microbial abundances. The most abundant species found in the control were Enterococcus, Akkermansia, Bacteroidales, Lactococcus and Vagococcus, to name a few. In PS-fed Tenebrio, abundance of Enterococcus sp. was shown to have significantly decreased while that of Vagococcus increased. The current study reports on metagenomic diversity and identification of major gut microbial species present in Plodia fed with Polyethylene only, compared to Plodia fed a normal diet of bran. Microbial DNA extracted from these larvae was sequenced by targeting the v3 and v4 regions of 16S rRNA. Qiime and Usearch analysis pipelines helped identify the microbial species along with statistical tests to determine any significant changes in abundances. Using Qiime, we show that the following microbial groups significantly increased in those larvae with PE as their sole source of food: Tepidimonas, Pseudomonas, Rhizobiales and Methylobacteriaceae. On the contrary, a sharp decrease in abundance of Firmicutes, specifically Turicibacter, indicated that they might not be involved in the polyethylene degradation process. Results from this study contribute to understanding of the functionality of this mixed community that enables it to degrade PE successfully. Applications for plastic recycling, one of the most intractable problems of human society today, are expected deliverables from this study.
Background: Heavy metal contamination in soil, due to modern industrialization, has emerged as serious global concern. The non-degradable properties of heavy metals allow them to exist in environment for longer durations. Chromium is a potent heavy metal pollutant which is widely used in industrial settings including paint industry, leather tanning and metal finishing industry. Chromium is a non-essential metal for various life forms and has been reported to persist in various habitats due to excessive industrial consumption. The Chromium hexavalent ion Cr (VI) has been found to cause breathing complications like nasal irritation as well as ulceration, skin irritation, eardrum perforation which subsequently leads to lung carcinoma. By employing chromium reduction potential of microbes along with chromium scavenging ability of hydrophytes, a powerful strategy can be established to cope with increasing chromium contamination.

Methods: In this study, Bacterial strains were isolated from samples collected geothermal springs of Chillas, Gilgit Baltistan, Pakistan. The metal resistance profile of isolates was determined against Cr-VI, Arsenic, Zinc and Manganese. The genomic DNA of selected microbes was extracted and sequenced. Among all isolates, Bacillus cereus TA2 and Bacillus cereus TA4 were selected owing to their ability to withstand high temperatures up to 45oC. The chromium tolerance potential of both TA2 and TA4 was estimated to be 500 µg ml⁻¹ and 600 µg ml⁻¹ K₂CrO₄, respectively. This ability to tolerate chromium at high concentrations made these strains a strong contender for plant-microbe interaction with Lemna minor.

Results: The association of TA2 and TA4 with Lemna minor raised levels of chromium reduction by these strains. Moreover, in Lemna minor, the contents of acid phosphatase, soluble proteins and peroxidase were found to be increased. However, the pigment content in Lemna minor was reduced. The bacterial strains enhanced chromium uptakes capabilities of Lemna minor.

Conclusions: The ability of Plants and microbes to coexist in order to increases chances of survival has opened an avenue of immense research for science to exploit this potential for mankind’s welfare. This association has promising potential to deal with metal contaminated habitats and industrial settings. The microbe-assisted-phytoremediation can be efficiently utilized for the decontamination of Chromium contaminated areas.
Do Microbes Have Memory? Repeated Exposure to Emulsified Vegetable Oil May Increase Degradation Ability of Native Microbial Communities

Background: Microbial “memory response” is the idea that microbial communities will degrade a substrate more rapidly if it has been exposed to it multiple times. This novel idea has the potential to increase the efficiency of many commonly-used bioremediation techniques. In order to test this concept, anaerobic microcosm experiment was conducted for 150 days using sediment and groundwater from a low-contamination aquifer at the Oak Ridge Field Research Center which had been previously amended with an emulsified vegetable oil (EVO) injection years before. Methods: Four groundwater wells from the same site were used to create the microcosms—two of the wells were directly downstream from the previous injection of EVO, and the other two were upstream and unexposed to EVO. All microcosms were amended with EVO, and changes in both microbial communities and geochemical properties were compared to see if the rate of degradation was faster in those that had already been exposed to EVO. Gas chromatography was used to measure CO2 and CH4 production in the microcosms at several time points, while ion chromatography measured levels of acetate, nitrate, and sulfate in the water. ICP-MS was also utilized to measure trace metals found in the water and sediment. To analyze microbial communities, DNA was extracted from both microcosm sediment and groundwater followed by 16S rRNA sequencing. Results: Results showed that after EVO addition, CH4 and CO2 were produced in both upstream and downstream samples at the same rate; similarly, nitrate and sulfate were also consumed at the same rate. However, acetate formed by EVO degradation was produced more rapidly and in much higher abundance in downstream wells. 16S data indicated that the relative abundance of known sulfate-reducing taxa, including those from the family Desulfobacteraeae, Desulfovibrionaceae, Geobacteraceae, and Desulfobulbaceae, increased and peaked around 30 days after EVO amendment, however, abundance was higher in downstream samples. Detrended correspondent analysis of OTU tables show that throughout all time points, there is a significant difference in the taxonomic community and population relative abundances between upstream and downstream wells at each timepoint. Conclusions: This data indicates that perhaps degradation occurs at the same rate in both previously exposed and un-exposed samples, however the abundance of relevant degrading-species—and therefore overall reduction ability—may be higher in the previously exposed samples.
Evaluation of the Diffusion Chamber and Microbial Trap Approach to Isolate Uranium and Nickel Resistant Soil-Borne Biodegradative Microorganisms

Primary Author Block:
R. Jaswal, A. Pathak, B. Edwards, III, D. Chappell, A. Chauhan; Florida A&M University, Tallahassee, FL

Abstract Body:
Microorganisms underpin most of the biogeochemical transformations in the environment including remediation of toxic contaminants. However, less than 1% of microbes can be cultivated and isolated under standard lab conditions. Towards this end, we evaluated the use of diffusion chamber (DC), and microbial trap (MT) approach for the cultivation and isolation of Uranium (U) and Nickel (Ni) resistant bacteria from historically contaminated environments. Soils were collected from the Steed Pond location at the Savannah River Site (SRS), SC which remains co-contaminated by radionuclides, metals and organics from the nuclear era weapon production activities. Briefly, soils are diluted with sterile water, combined with sterile agar, inoculated into a chamber that is lined by membrane filters and placed on wet soils; this permits the diffusion of nutrients into the chamber and facilitates growth of environmental microbiota within the agar. We established DC with the membrane pore size of 0.03 µm on both sides of the chamber. Simultaneously, MT were also established such that the top membrane was of a pore size of 0.03 µm and the bottom membrane, which is in contact with the soils, is of 0.2 µm size. In the MT, soil actinomycetes enter the agar through the bottom 0.2 µm size membrane and are ‘trapped’ in the chamber. After incubating for 20 days, colonized biomass from within the agar plugs were spread onto LB amended with U and Ni to isolate and identify potentially novel biodegradative microbes. A part of the biomass was also transferred into new plugs and processed for 2-3 cycles; biomass after each cycle was serially diluted and plated onto LB with U and/or Ni for microbial enumeration. 16S gene based identification of the DC and MT isolated strains revealed Burkholderia sp. to be the most abundant U and Ni resistant microbiota. Also obtained were two Penicillium strains using only the MT approach, which were previously not isolated by the traditional culturing. Interestingly, amplicon based metagenomic analysis also confirmed that Burkholderia spp., and Penicillium spp., were the most abundant bacterial and fungal genera in the soils used for establishing the diffusion chambers. Statistical analysis indicated that the microbial diversity was considerably different between the DC and MT approaches when compared to plate cultures, suggesting that this approach can be successfully applied to isolate metal resistant microbiota which can then be studied for their environmental remediation abilities.
Abstract Title:
Effects of Military Relevant Chemical Contaminants on A Reptilian Model Species Microbiome

Primary Author Block:
K. Indest, C. Jung, S. Everman, S. Newell, J. Lindsay; U.S. Army Engineer Res. and Dev. Ctr., Vicksburg, MS

Abstract Body:
Background: While mammalian microbiome-based studies have become ubiquitous in the medical literature, there have been limited gut microbiome studies focused on ecologically relevant vertebrate models like reptiles. Because of their relatively small home range, fast maturation, and high fecundity, lizards are an excellent reptilian terrestrial indicator species. For this study we used the green anole, Anolis carolinensis, as our model lizard. Anoles are commonly found on DoD installations in the southeastern US, and as a result have been used to assess toxicity of military relevant contaminants. We hypothesize that predictable changes in lizard gut microbiome composition, as a result of contaminant exposure, may serve as an easily assayed, noninvasive biomarker for a chemical exposure. Methods: Fourteen day sub-acute exposures were conducted with 2,4,6-Trinitrotoluene (TNT) at a dose of 60 mg/kg of body weight. Anoles (n=7) were orally gavaged daily using corn oil as a carrier with controls only receiving soybean oil. Body weights and food consumption were monitored and fecal samples were collected for high-throughput 16S DNA sequencing and analytical chemistry at days 0 and 14. At the end of the study, organs were harvested for body burden data. Microbial community sequence analysis was accomplished using the QIIME pipeline. Results: Significant changes in lizard weight loss (~6%) were observed in the TNT dosed anoles at completion of the experiment. Chemical analysis confirmed accumulation of TNT and TNT transformation products in tissue and fecal samples. PERMANOVA analysis of control and TNT bacterial fecal communities revealed significant differences at the end of the study with members of the Enterobacteriacea enriched for in the TNT dosed lizards. Conclusions: Previously, members of the Enterobacteriacea have been shown to transform TNT as a result of nitroreductase activity. Such activity may have enriched for these organisms in the fecal microbiome. Predictable changes in lizard gut microbiome composition could offer an easily assayed, noninvasive biomarker for a specific chemical exposure providing enhanced scientific support to risk assessments on military installations.
Targeting Microbial Arsenic Resistance Genes: A Meta-Analysis Across Soils

Primary Author Block:
T. K. Dunivin, S. Yeh, A. Shade; 1Michigan State Univ., East Lansing, MI, 2Univ. of Maryland, College Park, MD

Abstract Body:
Arsenic is a ubiquitous and toxic metalloid, and its biogeochemical cycling is impacted by microbial metabolism. Despite extensive culture-dependent studies, the biogeography and diversity of arsenic resistance genes is under-characterized. We developed an open-access pipeline for testing different sequencing datasets for nine arsenic resistance genes: acr3, aioA, arsB, arsC (grx), arsC (trx), arsD, arsM, arrA, and arxA. Our pipeline includes BLAST databases, hidden markov model searches and gene-targeted assembly. Using this pipeline, we examined the distribution and diversity of arsenic resistance genes in soil. We first tested all genomes in the RefSoil database for arsenic resistance genes. 85.14% of all RefSoil genomes tested positive for at least one arsenic resistance gene. The most common genes detected were acr3, arsB, arsC (grx), and arsC (trx). To further investigate the diversity and abundance of arsenic resistance genes in soil, we used a gene-targeted assembler to test 39 public soil metagenomes from the USA, Malaysia, Russia, Brazil, and Canada for arsenic resistance genes. Coverage-adjusted abundance for each gene was normalized to single-copy gene rplB for comparison. Arsenic resistance genes were present in all soils tested; however, differences in genetic potential for arsenic metabolism was observed between sites. Thus, despite the ubiquity of microbial arsenic resistance genes, microbial communities differ in their potential to impact arsenic biogeochemical cycling. Furthermore, phylogenetic analysis revealed novel diversity of dissimilatory arsenic metabolism genes (aioA, arrA, arxA). Thus, our pipeline not only allows the biomonitoring of arsenic resistance genes but also has the potential to extend the known diversity of arsenic resistance genes. Future work that uses this pipeline will offer a more complete understanding of the diversity and occurrence of arsenic resistance genes, which ultimately will provide insights into the ecology of microbial arsenic resistance.
Abstract Title:
Antibiotic Resistant E. coli, Including Esbl Containing Strains, from A Wetland Dominated by Crows

Primary Author Block:
K. Sen, T. Berglund, M. A. Soares, L. N. Khalil, Y. Ma, R. J. Turner; Univ. of Washington at Bothell, Bothell, WA

Abstract Body:
Free living birds can be significant contributors of antibiotic resistant bacteria (ARB). A constructed wetland, where ~15,000 American crows (Corvus brachyrhynchos) roost between autumn and spring, was sampled on the University of Washington Bothell Campus for the presence of Antibiotic resistant E. coli (ARE). Crow droppings from individual birds and grab samples of water were collected in 2014-2015 from different sites in the wetland influenced either by North Creek, campus run-off or surface water. E. coli were isolated from 49 of 61 samples by selective agar plating. Water samples were collected from these sites during 2016, to determine storm water contribution of ARE. A total of 98 fecal and 155 water E. coli isolates’ susceptibilities were tested against 13 antibiotics using the Kirby Bauer method. Antibiotic resistance (AR) to ampicillin (59%), amoxicillin-clavulanic acid (54%), streptomycin (49%), nalidixic acid (NA) (48%), neomycin (N) (38%), ceftiofur (19%) and tetracycline (Tc) (17%) was identified in the fecal isolates and ~ 20% were multidrug resistant (MDR). Water isolates displayed similar AR pattern but had less percentage of ARE relative to the feces; further testing is needed for verification. Water samples collected during storm events had ~ twofold increase in S, NA and (Tc) resistant E. coli. Tc resistant isolates frequently carried tet(A) (33%) or tet(B) (23%) genes. Presence of Extended Spectrum b-lactamase (ESBL) containing E. coli was determined using selective plating and verification of specific genes by PCR. The blaCTX-M gene was found in 16 water and 7 fecal isolates. blaCMY-2 gene was also present in 4 of the fecal isolates carrying blaCTX-M. All ESBL containing isolates were MDR, with 10 being resistant to NA, Tc and to S or N. Multilocus Sequence Typing analysis (MLST) identified three crow ESBL E. coli belonging to the internationally distributed ST131 clone. ST131 is highly virulent, and usually multidrug resistant; it is responsible for ~ 20% of the ESBL isolates globally. Two non-ESBL isolates with the exact same AR pattern, one each from water and feces, belonged to the human clone ST58. Phylogenetic analysis of the crow isolates by a PCR method, showed 37% to belong to the commensal strain phylo-group B1, and included the ST58 isolates. The B2 group, to which most extra-intestinal pathogenic E. coli belong, was the next most common (21%) and included the ST131 strains. This study demonstrates that American crows can acquire human associated ARB, including virulent ESBL strains, and act as reservoirs for these strains.
Abstract Title:
Longitudinal Field Study Evaluating the Spillover of Antibiotic Resistant Escherichia coli from Poultry to Humans in Ecuador

Primary Author Block:
H. Hedman1, J. Eisenberg1, G. Trueba2, D. Vinueza River2, R. Zurita Herrera2, J. Villacís Barrazueta2, L. Zhang3, S. Meda4, G. Gavilanes5; 1Univ. of Michigan, Ann Arbor, MI, 2Univ. San Francisco de Quito, Quito, Ecuador, 3Michigan State Univ., East Lancing, MI, 4Michigan State Univ., East Lansing, MI, 5Univ. San Francisco de Quito, East Quito, Ecuador

Abstract Body:
Small-scale farming operations in rural communities often prescribe high amounts of antibiotics for industrial meat production breeds of chickens (e.g. broilers). In contrast, free-ranging local varieties of backyard chickens receive almost no antibiotics. Recent evidence suggests that backyard chickens in proximity to broiler chickens have increased levels of phenotype and genotype antibiotic resistance. We conducted a seven-month longitudinal study aimed to examine whether backyard chickens and children serve as sentinels for detecting antibiotic resistance spread into the environment from broiler chickens in northwestern Ecuador. Escherichia coli isolates were identified from children (n = 1144), backyard chickens (n = 1323), and 1-day-old broiler chickens purchased from vendor sources (n = 253). Isolates were examined for their resistance phenotypes to 12 antibiotics and selected resistance genes. Phenotype resistance profiles fluctuated over time for human and backyard chicken samples. In contrast, broiler chicken resistance profiles remained high for all antibiotics tested. We also detected that households closest to households raising broiler chickens yielded significantly greater phenotype resistance levels among avian and human samples (general additive model; p < 0.005). The same blaCTX-M gene was detected in both human and chickens. These results likely suggest that small-scale broiler farming operations may function as sources of environmental antimicrobial exposure for the surrounding human and animal populations. Our results indicate that industrial meat-producing animals may introduce antibiotic resistance into other animal breeds, likely through horizontal gene transfer spillover events into backyard breeds and humans.
Antimicrobial resistance (AR) has emerged as a serious public health threat. The Centers for Disease Control and Prevention reports that AR infections account for two million illnesses and 23,000 deaths annually in the US, with over $20 billion as direct health-care costs and $35 billion in lost productivity. The National Antimicrobial Resistance Monitoring System (NARMS) was established as a surveillance system to track changes in antimicrobial susceptibility of enteric bacteria found in ill people, retail meats, and food animals. With animals being recognized as a reservoir of AR bacteria, animal-derived foods represent a major source of these bacteria to humans. Thus, there is a need to determine the prevalence of AR bacteria in animal-derived foods, and delineate their AR profile for devising appropriate control strategies. Escherichia coli and enterococci have emerged as important nosocomial pathogens in humans, with food suggested as a potential source. Although studies have indicated the prevalence of AR E. coli and enterococci in a variety of meats, no reports are available in the northeast. This study screened 202 samples of retail meats, including 80 chicken, 51 ground turkey, 36 ground beef, and 35 pork chops procured between 2016 and 2017 from commercial markets in three Connecticut counties for enterococci and E. coli. NARMS procedures were followed for the isolation and biochemical identification of the isolates, which were further confirmed by Matrix Assisted Laser Desorption/Ionization technology. Enterococci was isolated from 151 (74%) retail meat samples, with increased prevalence in ground meats (95%). E. faecalis (82%) was the most commonly isolated enterococci, followed by E. faecium (11%). E. coli was isolated from 58 (28%) of the retail meats, with greater isolation from retail poultry (53%). Over half of the E. coli were resistant to tetracycline, and one-third were resistant to aminoglycosides and penicillin. Antibiotic-resistant E. coli was most frequently isolated from ground turkey (67%) compared to other meats. Nearly 95% of enterococci isolates were resistant to at least one antibiotic and 55% were multi-drug resistant (resistant to 3 or more drugs). These isolates were resistant to lincosamide (92%), streptogramin (84%), tetracycline (58%), aminoglycosides (18%), quinolones (1.3%), and vancomycin (<1%). Results indicate a high prevalence of AR enterococci and E. coli in retail meats sold in Connecticut, and justify continued surveillance to monitor AR bacteria in meats from other counties in the state.
Abstract Title: Parallels among Culturable Antibiotic Resistant Fecal Coliforms and Resistance Genes from Soils Amended with Dairy Manure Or Compost During Vegetable Cultivation

Primary Author Block:
L. Wind, L-A. Krometis, W. Hession, A. Pruden; Virginia Tech, Blacksburg, VA

Abstract Body:
Identification of agricultural practices that mitigate the environmental dissemination of antibiotic resistance is a key need in preserving drug efficacy and protecting public health. We evaluated the effects of soil amendment type (inorganic fertilizer, raw dairy manure, composted dairy manure, or no amendment), vegetable type (lettuce, radish), and antibiotic use (pirlimycin and cephapirin) of cattle manure-derived amendments on the incidence of culturable antibiotic-resistant fecal coliforms through a field-scale controlled plot experiment. To reduce statistical bias associated with values below the limit of detection, zero-inflated Poisson (ZIP) regression models were used to identify significant trends in coliform count data. Antibiotic-resistant culturable fecal coliforms were recoverable from soils across all treatments immediately following application, though persistence throughout the experiment varied by antibiotic class and time. Compost-amended soils had the highest levels of cephalosporin-resistant fecal coliforms (5.64 log10 CFU/ g of soil), regardless of the antibiotic history of the cows providing the manure. Significantly, higher levels of total, ceftazidime, and erythromycin-resistant fecal coliforms were recovered from compost-amended as compared to the raw manure-amended soils (p < 0.01). Parallel quantification of resistance genes (sul1, tet(W), erm(B), intI1) was used to confirm observed culturable trends. Soils amended with raw dairy manure yielded high relative sul1 and tet(W) gene copies on Day 0, correlating with an observed spike in associated ARBs, and remained detectable for 113 and 39 days longer than resistant bacteria, respectively. Interestingly, erm(B) was not detected, despite detection of erythromycin-resistant bacteria throughout the experiment. This work is of particular interest given the relevance of fecal coliforms in tracking human pathogen risk in multiple environments (e.g., water, crops) throughout agricultural production.
Session Number: 151
Session Type: Rapid Fire
Session Title: Resistance is Not Futile! Antibiotics and AMR in Ag and the Environment
Session Start Date Time: 6/8/2018 3:00:00 PM
Session End Date Time: 6/8/2018 3:45:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 9129
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Christina Nishimura; 1
Abstract Body:
Screening of Soil Microbial Isolates for Antibiotic Activity

Primary Author Block:

H. Sahinoglu, P. Thomase, N. Daniels, C. Ingram, B. Okeke; Auburn Univ. at Montgomery, Montgomery, AL

Abstract Body:

Antibiotic resistance by pathogenic microorganisms spurred increasing research on new antibiotic substances. Reasons that account for antibiotic resistance by microorganisms include genes for enzymes that inactivate antibiotics, ejection of the antibiotic by plasma membrane proteins, and mutations affecting mode of action of the antibiotic. This research attempts to isolate potent antibiotic producing bacteria from soil. The soil samples were collected from Prattville, AL, North-Montgomery, AL, and around the AUM campus in Montgomery, AL. Three random soil samples were collected from each area and pooled. Nineteen tentative antibiotic producing isolates were purified by repeated streaking on tryptic soy agar plates. After further screening by agar plate assay, four isolates, N-1, P-2, P-12, P-13, were selected as antibiotic producers. Isolate P13 strongly inhibited Staphylococcus aureus and S. epidermidis and slightly inhibited Citrobacter freundii and Alcaligenes faecalis on agar plates. DNA based techniques were employed to characterize the selected isolates. BLAST analysis of ribosomal RNA gene sequence of P-13 revealed similarity to Bacillus amyloliquefaciens, B. siamensis and B. methylotrophicus. Production of antibacterial substance by P-13 in liquid culture is being studied.
Session Number: 176
Session Type: Rapid Fire
Session Title: Pathogens in the Environment and How to Get Rid of Them
Session Start Date Time: 6/8/2018 4:30:00 PM
Session End Date Time: 6/8/2018 5:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 8773
Poster Board Number:

Abstract Title:
Prevalence of Mycotoxins in Selected Nigerian Fermented Foods

Primary Author Block:
A. Obadina1, I. Olotu2; 1Federal Univ. of Agriculture, Abeokuta, Nigeria, 2Univ. of Johannesburg, Johannesburg, South Africa

Abstract Body:
Background: In Africa, fermented foods and beverages play a significant role in contributing to food security and their production is dominated by informal processing sectors that make use of different traditional processing methods which affects the quality and safety of the final products. This study evaluated the safety quality in terms of mycotoxin prevalence of some fermented foods obtained from Nigerian markets. Methods: Fermented food samples (n = 191) including maize gruel (ogi), sorghum gruel (ogi baba), melon seed (ogiri), locust bean (iru) and African oil bean seed (ugba) from Southwest Nigeria were quantified for 23 mycotoxins, including aflatoxin B1 (AFB1), fumonisin B1 (FB1), and sterigmatocystin (STE) using liquid chromatography-tandem mass spectrometry. Results: Data obtained revealed that 82% of the samples had mycotoxins occurring singly or in combination. FB1 was present in 83% of ogi baba samples, whereas 20% of ugba samples contained AFB1 (range: 3 to 36 µg/kg) and STE was present in 29% of the ogi samples. Ochratoxin A was found in ogi baba, iru, and ugba, at mean levels of 6, 6, and 9 µg/kg, respectively, whereas, roquefortine C was only detected in iru and at a low incidence rate (range: 10-14 µg/kg). HT-2 was the most frequently occurring trichothecenes in the samples and the level of deoxynivalenol in all the positive samples (n = 18) reached a maximum of 118 µg/kg. In terms of multi-mycotoxin contamination, FB1 + FB2 + FB3 + STE + AFB1 + alternariol + HT-2 co-occurred within one sample. Conclusions: This is the first study to report a wide range of previously unreported mycotoxins in iru, ogiri and ogi consumed in Nigeria. The extent to which the analysed mycotoxins contaminated these food commodities justifies the need to enact fungal and mycotoxin mitigation strategies along the food chain.
Abstract Title:
Prevalence of Shigatoxin-Producing Escherichia coli in Different Types of Flour
Primary Author Block:
S. Schlager, C. Schlagenaufen, S. Neubauer, K. Hauser, E. Edler, B. Springer, F. Allerberger; Austrian Agency for Health and Food Safety (AGES), Graz, Austria
Abstract Body:
Background: Two recent outbreaks of Shigatoxin-producing Escherichia (E.) coli (STEC) infections, one centered in the United States and one in Canada, were caused by contaminated flour [1,2]. We studied the prevalence of STEC in flour from Austrian retailers. Methods: Thirty-one different types of flour (22 produced from wheat, five from rye and four from spelt) were obtained in five Austrian groceries in July 2017. They originated from seven different mills and were tested for the presence of STEC using ISO/TS 13136:2012. In short, organisms were cultured in buffered pepton water for 24 h and PCR was used to test for stx1 and stx2. In case of positivity viability of STEC was tested on SMAC and TBX agar. For the confirmation of STEC in human samples primers and probes published by Reischl et al. (J Clin Microbiol. 2002; 40:2555-65) were used. Shigatoxin (stx) subtyping was performed in accordance with the PCR scheme of Scheutz et al. (J Clin Microbiol. 2012; 50:2951-63). Results: Six out of 31 flour samples (19.4%) were stx-positive after enrichment (3x wheat, 2x rye and 1x spelt). In five of these six samples we detected stx2g and in one wheat flour sample stx2b. Viable STEC were detectable in four of the six positive samples. While we failed to isolate purified STEC in three specimens, one of the four culture-positive samples yielded a strain of STEC O36:Hrough. In contrast, none of 212 STEC-positive human fecal samples (collected from July to December 2017) available to the Austrian Reference Center for E. coli yielded detectable stx2g; 37 of these 212 fecal samples tested positive for stx2b (17.5%).
Conclusions: Both stx2b and stx2g are genes considered to exhibit only low virulence. We were unable to identify the presence of high virulence stx2a, stx 2c or stx 2d in flour, and so far no Austrian cases of STEC infections have been epidemiologically linked to flour or consumption of raw dough. Our findings indicate that flour may pose a risk for STEC infections also in Austria.
Covert Rift Valley Fever in the Domestic Ruminant Populations in Uganda


Abstract Body:
Prior to the first recorded outbreak of Rift Valley Fever (RVF) in Uganda of March 2016, studies indicate presence of the RVF virus in Uganda without any overt outbreaks in either man or animals. Additionally, a number of isolates were also obtained, including the primordial isolate, from Semuliki Forest the mosquitoes of the Eretmapodite spp, for the Smithburn Modified Live Virus Vaccine (SMLVV) for animals. The first 2 datasets in this paper provide recent evidence, before March 2016, of the RVFV in Uganda, in select areas in the domestic ruminant populations. In 2013, sero-survey specimens from the districts of Hoima, Kibaale and Masindi were analyzed using a RVF inhibition ELISA and the results show an overall prevalence of 18.6% (12.5-26.7%) in the cattle population and 2.3% (0.4-12.1%) in the shoats population. In cattle, the prevalence was 12.1% (4.8-27.3%), 10.0% (2.8-30.1%), and 25.0% (15.8-37.2%) in the districts of Hoima, Kibaale and Masindi respectively while in shoats the prevalence in Masindi was 3.1% (0.5-15.7%), no antibodies could be detected, in shoats, in Hoima district. During an investigation in Agago and Kitgum districts of an undiagnosed illness in humans in 2011, a RVF sero-prevalence of 4.7% (1.3-15.5%) was detected in cattle while in shoats, it was 9.4% (3.2-24.2%). Both IgG and IgM antibodies to the RVF virus were detected at that time. These findings are synonymous to occurrence of disease during the ‘inter-epizootic periods’ in countries experiencing cyclic outbreaks. After the March 2016 RVF outbreak in Kabale, a planned multi-sectoral bio-surveillance pilot study was conducted in the outbreak district of Kabale and the surrounding ones. The study districts included; Kabale, Kanungu, Kasese, Kisoro and Rubirizi with the respective percentage (Positives / Animal numbers) data of 16.0% (83/520), 2.1% (4/193), 0.8% (1/130), 15.1% (21/139) and 2.7% (4/148). Of the 3 species investigated, the bovines exhibited the highest sero-prevalence of 15.2% followed by ovines with 5.3% and caprines with 4.0%. The study results are consistent with the confirmed outbreak in Kabale district and suspect cases in Kisoro district, in the domestic ruminant populations, around the same time period. The latter study is the most recent attempt to determine RVF sero-prevalence in Uganda, in many years. A more detailed study has been designed aimed at mitigating the risk of RVF in human and animal populations.
Abstract Title:
Exposure to Manuka Honey Modules Antibiotic Susceptibility on Wound Isolates

Primary Author Block:
J. Mokhtar, A. J. McBain, R. G. Ledder, G. Humphreys; Univ. of manchester, Manchester, United Kingdom

Abstract Body:
Background: The clinical application of Manuka honey has recently gained momentum, particularly with regards to the treatment of chronic wound infections. Changes in antibiotic susceptibility have been observed previously, following the exposure of bacteria to subtherapeutic concentrations of honey, however such findings have been limited to Methicillin-resistant Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas aeruginosa. The aim of this study is to assess the modulation of antibiotic sensitivity in a broader panel of chronic wound isolates.

Methods: Parent strains (P0) of Staphylococcus aureus, MRSA, S. epidermidis, Streptococcus pyogenes, P. aeruginosa, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis and Bacteroides fragilis were passaged ten times in the presence of sublethal concentrations of clinical grade Manuka honey to generate strain P10. In order to assess any permanent or transient changes in bacterial susceptibility, the bacteria were grown in honey-free media for a further 10 passages (X10). Antibiotic sensitivity testing was performed using a combination of microdilution and disc diffusion methodologies in order to determine MIC, MBC and MBEC values.

Results: Variable changes in bacterial susceptibilities were noted following subtherapeutic exposure to honey. P10 strains of S. aureus, S. epidermidis and S. pyogenes exhibited a ≥4-fold decrease in their sensitivities to erythromycin and tetracycline in comparison to baseline values. Similarly, P. aeruginosa displayed a 16-fold reduction in susceptibilities to both ciprofloxacin and gentamicin following passaging with honey. In contrast, K. pneumoniae and P. mirabilis showed notable increases in susceptibility towards both ciprofloxacin and gentamicin after 10 passages in the presence of honey. All changes in MIC, MBC and MBEC were shown transient in nature with the exception of P. aeruginosa (X10), which exhibited an MIC to ciprofloxacin >4 fold greater than the parent strain.

Conclusion: Wound isolates exposed to clinical grade Manuka honey exhibited transient changes in antibiotic profiles. The underlying mechanism and clinical implications of such changes are unclear and warrant further investigation.
Session Number: 176
Session Type: Rapid Fire
Session Title: Pathogens in the Environment and How to Get Rid of Them
Session Start Date Time: 6/8/2018 4:30:00 PM
Session End Date Time: 6/8/2018 5:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 9130
Poster Board Number:

Abstract Title:
MODERATOR

Primary Author Block:
Robab Katani; Penn State Univ., University Park, PA

Abstract Body:
Abstract Title:
Identification of Novel Compounds that Affect Bacillus anthracis Germination Induction

Primary Author Block:

Abstract Body:
Background: Our goal is to develop novel decontamination strategies that can be used after an accidental or intentional release of Bacillus anthracis spores. Bacterial spores are resistant to common methods of inactivation and decontamination. However, B. anthracis spores become significantly more vulnerable to stressors such as radiation, desiccation, and antimicrobials upon germination. The resulting germinated spores are highly susceptible to secondary treatment with common disinfectants.

Methods: In this study, we performed a high-throughput screen of 30,000 compounds in order to identify compounds that enhance germination in the presence of the germinants L-alanine and inosine (AI). Using a fluorescence-based kinetic germination assay, we have identified and individually confirmed multiple compounds that increase the germination of B. anthracis spores. Results: Compared to spores in AI, spores in Compound+AI mixtures show significantly more germination after one hour. We have evaluated the effect of these compounds on alanine racemase activity and we have assessed the level of antimicrobial activity in order to learn more about the mechanism associated with these compounds. Structurally, similarities exist between compounds that seem to affect B. anthracis spore germination such as the presence of hydroxyl quinolinone carboxamides. Conclusions: Together these data suggest the feasibility of using this high-throughput screening platform to identify compounds that enhance the germination of bacterial spores, and provide insight into molecules that enhance the germination of bacterial spores. Such compounds could improve wide-area decontamination by germination induction, which minimizes hazards to personnel and the environment associated with traditional methods of spore decontamination.
Session Number: 206
Session Type: Poster Talk
Session Title: Ready to Order? Microbes in Food and Drinks
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 12:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 8705
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Tatsuya Akiyama; 1
Abstract Body:
Abstract Title:
Rapid Method for Measuring the Effect of Prebiotics on Probiotic Bacteria Growth
Primary Author Block:
C. Oberg, D. Hoffman, M. Domek; Weber State Univ., Ogden, UT
Abstract Body:
Prebiotics are used to stimulate probiotic bacterial growth in the gut to optimize their health benefits. A rapid method was developed to evaluate potential growth enhancement by prebiotics on probiotic bacteria using a programmable spectrophotometer, standard microtiter plates, and commercial media, with growth enhancement results ready in 12 hours. Lactobacillus strains were grown in MRS broth while Bifidobacterium strains were grown in MRS broth with L-cysteine. Each culture was back diluted to an OD600 of 0.1 with the appropriate MRS broth then inoculated into wells (48 well plate) containing individual prebiotics. Plates were placed in a Tecan Infinite M200 spectrophotometer and incubated at 37°C with A600 readings taken for 12 h. Growth curves were done in triplicate with results compared to controls to determine extent of prebiotic growth enhancement. To optimize the method MRS concentrations of 20%, 35%, 50% and 100% were tested at selected pHs (7.0, 5.5, 5.0, 4.5, and 4.0) using 5 probiotic cultures. Addition of the bio-catalytic oxygen-reducing reagent oxyrase to test wells just prior to testing significantly enhanced growth of Bifidobacterium species and some lactobacilli such as Lb. acidophilus. Results indicated a 25% MRS broth at pH 5.0 with 2% oxyrase addition optimized prebiotic growth enhancement. Using this method, the stimulatory effect of added prebiotics (2% v/v) FOS, GOS, and XOS was determined for Bifidobacterium infantis M-63, Bifidobacterium longum BB536, and Bifidobacterium lactis BL-04, Lactobacillus rhamnosus LR-32 and Lactobacillus acidophilus NCFM. All three significantly improved growth of M-63, but only FOS increased growth of BL-04. For BB536, just GOS enhanced growth. GOS and FOS slightly improved growth of NCFM but no oligosaccharides enhanced growth of LR-32. The method allows rapid testing of various inoculum levels, prebiotic concentrations, media pHs, and prebiotic combinations for any probiotic strain including Bifidobacterium. With multiple samples run concurrently, comparisons can readily be made. In addition, the method can determine optimum enhancement of individual prebiotics or prebiotic combinations for any probiotic strain. 1
Abstract Title:
Cellulase Production from Penicillium Citrinum Using Pineapple Peels As A Cheap, Alternate Substrate
Primary Author Block:
O. Oyedeji; Obafemi Awolowo Univ., Ile-Ife, Nigeria
Abstract Body:
Cellulases are a major group of enzymes having wide range of industrial and biotechnological applications. The production cost of cellulase is a major factor limiting its use hence the need to develop low cost production systems for this enzyme. In this study, brewer’s spent grain and pineapple peels, which otherwise constitute sources of pollution to the environment, were investigated as cheap, alternate substrates for cellulase production from Penicillium citrinum, isolated from decaying orange fruits. Cellulase production was assayed by measuring the amount of glucose released in µmole per millilitre per minute by using the dinitro salicylic acid (DNS) method. Evaluation of process parameters affecting cellulase production was carried out. Cellulase titres $3.82 \pm 0.136$ U/ml and $1.405 \pm 0.151$ U/ml were produced by the fungus, using pineapple peels and brewer’s spent grain as substrates, respectively, under submerged fermentation. Maximum cellulase production from P. citrinum occurred with the use of pineapple peels as substrate, at 72 h fermentation period, with the use of pineapple peels at a concentration of 1.5%w/v and peptone as the best nitrogen source. The optimum temperature for the production of cellulase by the fungus was found to be 50 oC while the optimum pH was 6.0. Findings from this study indicated the potential use of pineapple peels as cheaper alternative substrate for the production of cellulase thus mitigating the hazardous effect it has on the environment as pollutant. P. citrinum was able to grow and produce good levels of cellulase using solely pineapple peels as low cost substrate, at high temperature of 50 oC, making this strain and this low cost agro-industrial residue worthy of further investigation and potentially feasible for wide-range of biotechnological applications.
Bioconversion of Potential Efficacy Compound in Medicinal Plants Through Lactic Acid Bacteria Isolated from Kimchi

Abstract Body:
The purpose of this study was to develop functional foods as a multi-effect anti-diabetic agent that work simultaneously on various targets through microbial conversion of compounds in medicinal plants. For the screening of strains with bioconversion activity, 280 strains of lactic acid bacteria (35 species in 5 genera including 2 novel species) were isolated from a traditional Korean fermented vegetable, kimchi. Among them, bioconversion was confirmed in the Lactobacillus spp., Enterococcus sp., Pediococcus sp., and Leuconostoc sp. through the submerged fermentation with medicinal plants. Representatively, Lactobacillus spp. or Enterococcus sp. demonstrated that initially abundant geniposide and gastrodin in the medicinal plants are converted into its corresponding aglycones, genipin and 4-hydroxylbenzyl alcohol, respectively, after the fermentation at 28 °C for 48 h without additional nutrient supplementation. Substances present as glycosides in medicinal plants can only be effective after metabolism through intestinal microorganisms. From this perspective, our results also confirm that the efficacy of substances in medicinal plants have increased through microbial bioconversion. The anti-diabetic effect of bio-converted compounds will be discussed in detail in this text.
Antimicrobial Activities of Cranberry Extracts against Salmonella enterica Serovars from Broiler Chicken

Abstract:

Background: Cranberry fruits possess antimicrobial properties due to its various acids and phenolics compounds, however, the underlying mechanism of actions are poorly understood. We evaluated the effects of cranberry extracts on growth rate and the transcriptome of Salmonella enterica serovars.

Methods: Two sub-fractions, anthocyanins (A20) and non-anthocyanin polyphenols (P85) from an ethanolic extract from cranberry pomaces (KCOH) were generated. The minimum inhibitory (MICs) and bactericidal (MBCs) concentrations of these fractions against S. enterica serovars Typhimurium, Enteritidis and Heidelberg were obtained using broth microdilution method (CAMHB) according to the CLSI’s guidelines. Transcriptional profiles of S. Enteritidis grown in cation adjusted Mueller Hinton broth (CAMHB) supplemented with or without 2 or 4 mg/ml of KCOH were compared by RNASeq performed on an Illumina MiSeq to reveal gene modulations serving as markers for biological activity. Results: The MIC and MBC of KCOH were 8 and 16 mg/ml, respectively against all tested S. enterica isolates, while these values were 8 and 4 mg/ml for A20 and P85 fractions, respectively. A20 and P85 induced up to 7 hours delay of growth initiation and a strong growth rate inhibition of Salmonella. Treatment of Enteritidis with KCOH revealed a concentration-dependent transcriptional signature. Exposure of Enteritidis at 2 mg/ml KCOH up-regulated expressions of 14 proteins, including phosphoenolpyruvate carboxylase, 4'-phosphopantetheinyl transferase (AcpT), pyruvate:ferredoxin (flavodoxin) oxidoreductase, multiple antibiotic resistance regulatory protein (MarB), Fe2+-enterobactin ABC transporter and SPI-2 type III secretion system effector (SpvB) and down-regulated expressions of 10 proteins. At 4 mg/ml of KCOH, expressions of 15 proteins including NarK family nitrate/nitrite MFS transporter were down-regulated while expressions of 5 proteins were up-regulated. Four genes were similarly modulated by both 2 and 4 mg/ml (two-component system response regulator DcuR, a membrane protein, a DUF91 domain-containing protein, and an anion permease) where anion permease down-regulated 6 -fold at 4 mg/ml in comparison to control. Conclusion: Cranberry constituents affect growth and modulate expression of genes associated with metabolic functions, osmolality, iron uptake and nitrate/nitrite transport in Salmonella.
Comparison of Raw Milk Microbiota in Cows with Staphylococcus aureus Positive and Negative Quarters

Primary Author Block:
M. Cyrenne1, D. Roy2, S. Dufour3, M. Chenier4, F. Malouin1; 1Univ. de Sherbrooke, Sherbrooke, QC, Canada, 2Univ. Laval, Quebec, QC, Canada, 3Univ. de Montreal, Montreal, QC, Canada, 4McGill Univ., Montreal, QC, Canada

Abstract Body:
Background: Staphylococcus aureus (SA) intramammary infection is an important cause of bovine mastitis. Dysbiosis (microbial imbalance) has been associated to some bacterial diseases. The aim of this study was to determine if any specific raw milk microbiota signature could be associated to a natural SA infection. Because microbiota are notoriously variable from one individual to another, we paid particular attention to the microbiota of SA positive and negative quarters within the same cow. Methods: Lactating cows from a commercial farm in Canada were first screened for the presence of SA in each quarter. To be selected, a cow needed to have one quarter with more than 100 CFU of SA/mL of milk, and one SA negative quarter. i.e., no SA colony for three consecutive samplings conducted at weekly interval. Four cows were selected and the microbiota of 8 quarters were analyzed. Milk was collected from those quarters and frozen on the same day. DNA was extracted using the PowerFood kit from Qiagen. Duplicates of samples were then amplified by qPCR with primers selecting for the V1-V2 region of the 16S RNA gene and barcoded before sequencing by Illumina MiSeq. Sequences were treated with the QIIME2 pipeline. As we wanted to analyze the impact of SA relative abundance on the milk microbiota, and because milk bacteriology tests may vary from time to time, samples were strictly classified according to the percentage of SA sequences detected (vs total sample sequences). A SA prevalence of 0 to 2% was considered a SA negative quarter (SA-) while SA+ samples had a SA prevalence that varied from 5 to 91%. Results: The richness and the diversity of the samples were evaluated with the Chao1 and the Shannon indexes. When comparing the microbiota of all SA+ samples to SA- samples, Shannon and Chao1 indexes were significantly lower in SA+ samples (p = 0.04 and p = 0.01, respectively). Individually, one cow stood out with a significantly lower Shannon index (p = 0.002) for its SA+ quarter vs its SA- quarter. That cow showed by far the highest milk somatic cell count (5 million vs 116-204×103 cells/mL for the other cows). Interestingly for all analyzed quarters, we observed an inverse correlation between the prevalence of Corynebacterium sp., Aerococcaceae, Staphylococcus hominis and Leuconostocaceae and the prevalence of SA. Conclusion: The presence of SA within the milk of a quarter can have an influence on other bacteria composing the microbiota, therefore modifying its diversity and richness. It remains to be seen if some bacterial species can play a protective role against SA.
Abstract Title:
E. coli Harboring the Colistin Resistance Gene, mcr-1, are Prevalent in Lebanese Poultry

Primary Author Block:
T. Sourenian, Z. Hmede, I. KASSEM; American Univ. of Beirut, Beirut, Lebanon

Abstract Body:
Antimicrobial resistance is a serious global problem. However, antimicrobial stewardship remains deficient in Lebanon. The latter is evident in unrestricted access to antibiotics without prescription to treat human ailments and enhance agriculture. Antimicrobial resistance monitoring programs are lacking, and data on the emergence of resistance are largely restricted to clinical studies. Furthermore, a review of current agricultural practices indicated that colistin is routinely used to control colibacillosis and salmonellosis in Lebanese poultry. As a part of an effort to establish a base-line for antimicrobial resistance in food and agricultural matrices in Lebanon, we investigated the emergence and prevalence of colistin resistance on Lebanese poultry farms. For this purpose, fresh fecal samples (n = 93) were collected from three major poultry farms located in North and South of Lebanon. All samples were initially screened on a selective medium, RAPID'E.coli 2 Agar, which was supplemented with 4 μg/ml of colistin. Our results showed that 90 samples (97%) yielded diagnostic E. coli colonies (~ 80 - 104 CFUs per ml). Sixty E. coli (20 per farm) were selected and purified and tested further for colistin resistance using the broth microdilution method and the presence of mcr-1 using PCR. Our results showed that the MIC for 82% of the isolates was greater than 8 μg/ml, while 95% of the isolates harbored mcr-1. Antimicrobial susceptibility analysis using the disk diffusion assay showed that the colistin resistant E. coli were also resistant to amoxycillin + calvulanic acid (79% of isolates), ampicillin (100%), cefepime (59%), cefalexin (91%), cefotaxime (72%), cefixime (77%), chloramphenicol (98%), ciprofloxacin (77%), gentamicin (52%), kanamycin (79%), norfloxacin (77%), penicillin (100%), streptomycin (77%), tetracycline (83%), and trimethoprim-sulfamethoxazole (90%). Taken together, our results show a notably high prevalence of resistance to colistin and other important antimicrobials in E. coli isolated from Lebanese poultry. mcr-1 was also widely distributed in these isolates. There appears to be a peremptory national need to revisit antimicrobial stewardship and agricultural practices in order to control the proliferation of antimicrobial resistance in Lebanon.
Abstract Title:
Municipal Wastewater as a Microbial Surveillance Platform for Enteric Diseases: A Case Study for Salmonella and Salmonellosis

Primary Author Block:
T. YAN1, J. Shelton1, P. O’Brien2, A. Whelen2, E. Pagaling1; 1Univ. of Hawaii at Manoa, Honolulu, HI, 2Hawaii Dept. of Hlth., Pearl City, HI

Abstract Body:
Municipal wastewater (MW) contains a conglomeration that includes the human enteric microbiome from a community, and hence represents a potential surveillance sample source for gastrointestinal infectious disease burden at the community level. To evaluate this, the concentration of Salmonella in MW samples from Honolulu, Hawaii was monitored over a 53-week period, which showed positive and significant rank correlation with clinical salmonellosis case numbers over the same period. Salmonella isolates were obtained from the MW samples, and then compared with clinical isolates submitted to the Hawaii Department of Health State Laboratories. The MW isolate collection contained 34 serotypes and the clinical isolate collection contained 47 serotypes, 21 of which were overlapping, including nine of the twelve most common clinical serotypes. There were nine Salmonella strains, including one outbreak-associated Paratyphi B strain, that were concurrently detected in health clinics and in the MW samples, indicating the feasibility of monitoring MW concentrations of enteric pathogens as a timely indication of community enteric disease activity.
Kitchen Towel As Risk Factor for Home Based Food Poisoning

S. Biranjia-Hurdoyal, V. Moodelly; Univ. of Mauritius, Reduit, Mauritius

Background: Cross contamination in the kitchen could contribute to home-based food poisoning. This study aimed at investigating the potential role of kitchen towels in cross contamination in the kitchen.

Methods: A total of 100 kitchen towels were collected after one month of use. The bacteria were cultured and identified by standard biochemical tests. A questionnaire was also designed to investigate the potential risk factors which could affect the result. Results: Bacterial growth was found in 49% of the kitchen towels and significantly increased by size of family, extended family and presence on children. Multipurpose towels had higher CFU than single use towels (1.31 x 10^7 vs 6.60 x 10^4; p<0.05) and humid towels had higher CFU than dry ones (4.8 x 10^5 vs 0.5x 10^5; p<0.05). The mean CFU from the towels was found to be 2.76 x 10^5 and was significantly higher from the cotton towels (4.98 x 10^5) compared to the nylon (1.64 x 10^5) and mixture of both towels (1.89 x 10^5). Out of the 49 samples which were positive for bacterial growth, 36.7% grew coliforms, 36.7% Enterococcus spp., 30.6% Pseudomonas spp., 28.6% grew Bacillus spp., 14.3% S. aureus, 4.1% Proteus spp., 2.0% coagulase negative Staphylococcus. Furthermore, S. aureus was isolated at higher rate from families of lower socio-economic status (p<0.05) and those with children (p<0.05). The risk of having coliforms was twice on humid towels than the dried ones. It was also noted that as the CFU increased, the detection rate of coliform, Enterococcus spp., Proteus spp. and Bacillus spp. also increased significantly. Furthermore, Enterococcus spp. and S. aureus were isolated at higher prevalence in bigger families (p<0.05). Diet was also found to be an important factor. Coliform and S. aureus were detected at significantly higher prevalence from families on non-vegetarian diets while a higher prevalence of Enterococcus species from the kitchen towels of vegetarian families. Conclusions: This study conclude that kitchen towels could be very important source bacterial contamination which could contribute to food poisoning. The multipurpose usage of kitchen towels should be discouraged.
Prevalence and Antimicrobial Resistance Escherichia coli Strains in Irrigation Canal, Dam, River, and Dike Water at the Northwest of Mexico

Background: Sinaloa a state located in the irrigated region of Northwestern Mexico, with a farming system that covers large tracts of arid lands across the northern parts of the country. It has several dikes along 12 dams and 11 rivers that are responsible for the irrigation of their farming valleys and also is the main producers of export-oriented products such as tomato, cucumber and mango. Recently, there is increasing evidence of the contribution of surface water in the contamination of food products leading to subsequent outbreaks of foodborne illness. Methods: The aim of this study was, therefore, to analyze surface water samples (n=472) collected from rivers (n=29), dikes (n=5), dams (n=9) and irrigation canals (n=429) for the presence of thermotolerant coliforms (also known as fecal coliforms) and diarrheagenic Escherichia coli (DEC) by PCR from January to December 2014. Results: Forty-seven percent (222/472) and 43.6% (206/472) of water samples were above permissive levels for thermotolerant coliforms and E. coli, detected by conventional bacteriology, respectively. Among 206 E. coli isolates, we molecularly identified 14% (29/206) as diarrheagenic E. coli (DEC) strains. These bacteria were isolated from different locations, Rivers samples (10.3%; 3/29) and Irrigation water (6.06%; 26/429) were the most contaminated samples with DEC, while DEC strains were not detected in dike and dam samples. Isolated DEC corresponded to entero-aggregative Escherichia coli (EAEC) 34.4% (10/29), entero-pathogenic Escherichia coli (EPEC) 31.0% (9/29), diffuse adherent Escherichia coli (DAEC) 27.5% (8/29), and enterotoxigenic Escherichia coli (ETEC) 6.8% (2/29) strains. Enterohemorrhagic Escherichia coli (EHEC) and Enteroinvasive Escherichia coli (EIEC) strains were not detected. Moreover, among isolated DEC strains, 88% exhibited resistance to at least one commonly prescribed antibiotic. Conclusions: The presence of potential diarrheagenic E. coli (DEC) and antibiotic resistance could represent a potential risk to human and animal health, and thus routine monitoring of DEC in surface water should be considered at northwest of Mexico.
Abstract Title:
Generating a Highly Attenuated Pseudomonas aeruginosa Strain

Primary Author Block:
M. Valentine1, T. R. Withers2, B. Kirby1, R. Niles1, H. Yu1; 1Progenesis Technologies, LLC, Huntington, WV, 2U.S. Food and Drug Admin., Morgantown, WV

Abstract Body:
Alginate is a commercially important polysaccharide that is extracted from brown seaweed. The utility of alginate lies in its biocompatibility and formation of sodium and calcium gels. The polymer is a common additive to food, cosmetics, and healthcare products. Climate change has decreased seaweed yields and alginate consistency, so there is a growing opportunity for more reliable sources of alginate. An attractive alternative for alginate production is Pseudomonas aeruginosa, a common Gram-negative bacterium that can form alginate-containing biofilms (mucoid phenotype). However, P. aeruginosa is an opportunistic pathogen that can cause serious infections in immune-compromised humans. The pathogenicity of P. aeruginosa can be attributed to several virulence factors and the ability to form a biofilm, which enables the organism to evade host defenses and antibiotics. To generate an attenuated P. aeruginosa strain useful for commercial production of alginate, we identified and deleted five key pathogenicity genes in the PAO1 strain: 1) toxA (exotoxin A), 2) plcH (exotoxin hemolytic phospholipase C), 3) phzM (pyocyanin), 4) wapR (lipopolysaccharide), 5) aroA (aromatic amino acid synthesis). We deleted these genes using a homologous recombination strategy with plasmid pEX100T, and confirmed them by DNA sequencing. Transformation of this strain (called PGN5) with a plasmid containing mucE, an activator of alginate synthesis, induces mucoidy indicating that the alginate biosynthetic pathway is functional. Our preliminary data suggest that these deletions reduce the pathogenicity of P. aeruginosa, and that the alginate produced by these strains is comparable to commercially available seaweed alginate. We are currently conducting studies to verify the attenuation of PGN5 by comparing its pathogenicity in mice to the Escherichia coli BL21 strain, which is FDA-approved. We are also determining the quantity, purity, and composition of the alginate produced by PGN5. Future studies will focus on creating a suite of P. aeruginosa strains that can produce alginate of different compositions to suit different industrial/biomedical needs.
**Abstract Title:**
Effects of Oxic-Anoxic Cycles on Microbial Community Biodegradation of Crude Oil in Beachsands

**Primary Author Block:**
P. Heritier-Robbins1, S. Karthikeyan1, M. Kim1, J. K. Hatt1, W. A. Overholt1, J. E. Kostka1, M. Huettel2, K. T. Konstantinidis1; 1Georgia Tech, Atlanta, GA, 2Florida State Univ., Tallahassee, FL

**Abstract Body:**
Following the 2010 Deepwater Horizon (DWH) blowout, large sections of the intertidal zones along the Gulf of Mexico became contaminated with crude oil slicks, which subsequently became trapped in sediments as tides receded. Oxygen levels in intertidal zone sediments mostly fluctuate due to the tides but the effects of these oscillations on microbial activity and oil-degradation rates remain essentially unknown. Advancing these issues will be important for better predicting and modeling microbial oil biodegradation in beachsands. The primary objectives of this study were to determine the effect of oxygen level oscillations on buried oil hydrocarbon degradation in the sediment and nitrogen (N) cycling, because N often is a limiting nutrient for oil biodegradation. For this, advective-flow chambers that mimic in-situ pore water exchange in saturated beach sediments were inoculated with weathered Macondo oil; un-inoculated chambers served as controls. Microbial oxygen consumption, coupled to oil biodegradation proceeded until anoxic conditions were reached. The chambers were then re-aerated to full oxygen saturation levels in order to simulate an oxic-anoxic cycle. The effects of the cycle on hydrocarbon degradation kinetics, nitrogen fixation and bacterial community shifts were quantified using mass spectrometry analysis for quantification of hydrocarbon depletion coupled with metagenomics and metatranscriptomics. Preliminary analysis revealed that the abundance of Alphaproteobacteria in oiled chambers followed closely the oxygen levels, and remained higher compared to the control chambers at all time points. Additionally, nitrogen fixation genes were found to significantly increase in oiled chambers, which coincided with an increasing rate of hydrocarbon biodegradation. This community succession pattern appeared consistent with that observed in field data from Pensacola beach following the DWH blowout. Reliable gene and taxa biomarkers of the different conditions were identified, which could be used by site managers to more precisely monitor the process of oil biodegradation in-situ.
Session Title: SATURDAY - AES Late-breakers

Abstract Title: Novel, Slow-Release Electron Donors for Chlorinated Solvent Bioremediation

Primary Author Block: K. T. Finneran, A. Rogier; Clemson Univ., Clemson, SC

Abstract Body:

Background: Chlorinated solvents account for approximately three quarters of all bioremediation sites. The vast majority of these remediation applications are predicated on a simple strategy: amend a high molecular mass electron donor into the subsurface so Dehalococcoides-like microorganisms are stimulated, and the activity is promoted over the long term. Thus far all long-term electron donors have been derivatives of soybean oil, which is problematic because of: a) limitations in the microbial populations that actually utilize strictly lipid electron donors, and b) competition with foodstuffs in US production. We have developed electron donors from rendered animal co-products, which are combinations of lipid, protein, and minimal carbohydrate. Thus far 21 co-products have been tested, and all stimulate complete dechlorination to a rate and extent, which is better than any current soybean oil-based electron donor. In addition, these materials are fractions of the cost of soybean-based commodity products, on the order of pennies per ton. Methods: Batch incubations were used to screen 21 animal co-products. Batches were constructed using TCE-contaminated aquifer material, and each electron donor (co-product) was added as the sole electron donor. Each animal co-product was compared to 5 controls containing common electron donors (lactate, acetate + hydrogen, and one soybean oil-based electron donor) and a sterile and unamended controls. TCE and its degradation products were quantified over time. Results and Conclusions: The data demonstrate that of the 5 controls, lactate was able to completely dechlorinate TCE in approximately 45 days. Lactate was the fastest of the 5 controls and as a result each animal co-product was compared to it. Of the 21 animal co-products, 17 completely dechlorinated the TCE to ethene at rates faster than lactate and 4 generated ethene at the exact same rate as lactate. In general, the more proteinaceous animal co-products were able to promote dechlorination at a faster rate than the animal coproducts with a higher fat content. All materials reduced TCE to ethene (at a 1:1 stoichiometry) faster than the commercially available soybean-based electron donor (e.g. emulsified vegetable oil). Dehalococcoides-like cells will be identified using QPCR, and future experiments will optimize the concentrations required to promote this activity. This strategy introduces a new electron donor for TCE bioremediation, which thus far is faster and more cost-effective than any electron donor reported to date.
Abstract Title:
Glycosylation and Subsequent Malonylation of Isoflavonoids in Engineered E. coli: Biochemical Engineering, Strain Development and Fermentor Scale Production

Primary Author Block:
N. Koirala; Dr. Koirala Res. Inst. for Biotechnology and Biodiversity, Kathamndu, Nepal

Abstract Body:
Background: Genistin and daidzein exhibit a protective effect on DNA damage and inhibit cell proliferation. Glycosylation and malonylation of the compounds increase water solubility and stability.

Methods: Constructed pET15b-GmIF7GT and pET28a-GmIF7MAT were used for the transformation of Escherichia coli and bioconversion of genistein and daidzein. To increase the availability of malonyl-CoA, a critical precursor of GmIF7MAT, genes for the acyl-CoA carboxylase α and β subunits (nfa9890 and nfa9940), biotin ligase (nfa9950), and acetyl-CoA synthetase (nfa3550) from Nocardia farcinia were also introduced. Results: Thus, the isoflavonoids were glycosylated at position 7 by 7-O-glycosyltransferase and were further malonylated at position 6″ of glucose by malonyl-CoA: isoflavone 7-O-glucoside-6″-O-malonyltransferase both from Glycine max. Engineered E. coli produced 175.7 μM (75.90 mg/L) of genistin and 14.2 μM (7.37 mg/L) genistin 6″-O-malonate. Similar conditions produced 162.2 μM (67.65 mg/L) daidzin and 12.4 μM (6.23 mg/L) daidzin 6″-O-malonate when 200 μM of each substrate was supplemented in the culture. Conclusions: Based on our findings, we speculate that isoflavonoids and their glycosides may prove useful as anticancer drugs with added advantage of increased solubility, stability and bioavailability. Moreover our approach of biochemical engineering paves the way for commercial production of isoflavonoid glucosides and malonates using microbial host system.
Abstract Title:
The Water-Filled Tree Hole: A Hypoxic, Reducing Environment with Novel Sedimentary Microorganisms

Primary Author Block:
J. K. Kirk, K. Kashefi, E. D. Walker; Michigan State Univ., East Lansing, MI

Abstract Body:
Aedes triseriatus and its water-filled tree hole habitat provide a model ecosystem for analysis of the factors constraining mosquito production. The tree hole ecosystem is heterotrophic, with trophic processes dependent upon microbially-mediated decomposition of organic detritus and inputs of inorganic nutrients from stemflow water. For growth in this ecosystem, mosquito larvae consume microorganisms. Our study challenges the assumption that tree hole production is nutrient-limited; instead, it is a nutrient-rich environment, but hypoxic, with a deficit of electron acceptors to further microbial respirations and decomposition, and a surfeit of electron donors establishing a reducing environment. This research elucidated understudied guilds of microorganisms dwelling in tree hole sediments whose anaerobic respirations involve electron acceptors other than oxygen to drive these decomposition processes. Using molecular and culture-dependent methods, we analyzed microbial communities and isolated novel sedimentary microorganisms, belonging to the families of Geobacteraceae and Clostridiaceae associated with tree hole sediment, to show that inorganic ions introduced largely through stemflow water provide a pulse of electron acceptors to microorganisms. These results, for the first time, highlight the importance of anaerobic microbial respirations in sediments of tree hole ecosystems, and provide novel insight on the importance of these microbial activities in controlling mosquito growth.
Abstract Title:
Simulation and Modeling of Dietary Changes in the Infant Gut Microbiome

Primary Author Block:
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Abstract Body:
Background: Early gut microbiome composition is essential for health in the short and long term. Diet is a key factor shaping the infant gut microbiome, and breast-fed and formula-fed infants have distinct gut microbiome patterns. Oligosaccharides in breast milk or formula act as prebiotics, influencing gut microbiome composition. Infants usually receive a mixed feeding regime of formula and breast milk, and this transition could influence microbiome composition.

Results: In this study we simulated the impact of a dietary switch from fructooligosaccharides (FOS) to 2-fucosyllactose (2FL) in a continuous culture system containing a consortium of species of the infant gut microbiome. Representative species used were Bifidobacterium longum subsp. infantis, Bacteroides vulgatus, Escherichia coli and Lactobacillus acidophilus. During growth on FOS the consortium was dominated by L. acidophilus, with the concomitant production of high amounts of lactate. Switching to 2FL led to an important decrease in total biomass and lactate in the bioreactor, and a recovery in B. infantis levels accompanied by an increase in E. coli. While FOS was rapidly metabolized by the consortium, 2FL first accumulated in the system and started to be consumed only 6 h later. 2FL consumption was followed by a gradual switch from lactate to acetate as major acids produced. The activity of the bacterial species correlated well with gene expression analysis, observing the induction of fructokinase in L. acidophilus during FOS culture, a fucosidase and galactosidase gene in B. infantis, and a fucose permease in E. coli during growth on 2FL. In addition, mathematical modeling of a multi-species consortium in a continuous culture with metabolic interactions was capable to explain in great part the behavior of the system. Moreover, the model was used to simulate the outcome of the system after 48 h after each regime, determining also that the system was able to return to a basal state after the dietary change.

Conclusions: These results provide important information to understand the impact of dietary changes in the gut microbiome, highlighting the relevance of transitions between these alterations. This work also provides an ODE-based modeling frame aimed to predict the impact of dietary alterations on the gut microbiome.
Abstract Title:
Genomic Characterization and Prioritization of Nitrogen-Fixing Bacteria Biofertilizers Isolated from Colombian Sugarcane Fields

Primary Author Block:
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Abstract Body:
Sugarcane (Saccharum spp.) are tall, perennial grasses cultivated in tropical and warm temperate regions in Colombia, South America. Sugarcane production and processing is a multi-billion dollar/year business, supporting food, energy, and other industries around the country. Previous studies have shown that sugarcane harbors diverse plant growth promoting microorganisms, including nitrogen-fixing bacteria, which have the potential to serve as biofertilizers. The use of biofertilizers in sugarcane agriculture is key to reducing dependence on environmentally damaging and expensive chemical fertilizers. We are collaborating with the Colombian sugar cane company INCAUCA to isolate and characterize native nitrogen fixing bacteria, with the aim of deploying them as plant growth promoting biofertilizers, and we previously isolated 23 nitrogen-fixing bacteria from INCAUCA sugar cane fields. The goal of this study was to use whole genome sequence analysis of these isolates in order to prioritize them with respect to their potential as biofertilizers. To this end, we are looking for strains that are predicted to have maximum benefit to the plants while presenting minimum risk to the environment, including local human populations. Functional genome annotations were used to prioritize strains that are enriched for nitrogen fixing and other plant growth promoting genes and depleted for virulence factors and antibiotic resistance genes. Comparative whole genome analysis revealed that 15 of 23 isolates belong to the genus Klebsiella, and 5 of 23 belong to genera closely related to Klebsiella. Functional annotation showed that all 23 isolates encode transcriptionally active nif operons, which are required for nitrogen fixation. These genomes also encode a variety of phosphate solubilization and siderophore production operons, as well as other genes involved in the synthesis of plant growth promoting metabolites (acetoin and butanediol). The isolates also contain antibiotic resistance genes (ampicillin, levofloxacin, and meropenem) and other virulence factors (host attachment factors and endotoxin). We developed a quantitative scoring system to rank potential biofertilizers by their predicted and experimentally validated growth promoting phenotypes, potential environmental risks, and antibiotic resistance profiles. Future work will be done to experimentally validate predicted biofertilizer activity along with potential virulence and antibiotic resistance of these isolates.
Development of Potent Bacteria-Based Formulation for Enhanced Bio-Control Potential against Bacterial Leaf Blight Pathogen in Rice

Primary Author Block:
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Abstract Body:
Globally, rising awareness of the hazardous effects of synthetic pesticides has increased the demand for safer alternatives. Biofertilizers manufactured in Pakistan are mostly carrier-based, and are reported to have short shelf life and inconsistent field performance. This study was carried out to formulate growth promoting biocontrol agents for the suppression of Bacterial leaf blight (BLB) in rice. The research was directed at basic and applied aspects of using beneficial bacteria as formulated product. Rhizospheric samples collected from major rice growing areas of Punjab were used for the isolation of bacteria antagonistic to BLB pathogen i.e. Xanthomonas oryzae pv. oryzae (Xoo). Of 230 different isolates, nine bacterial isolates showed antagonistic activity against Xoo. Pseudomonas sp. ZA86 showed the maximum inhibition of Xoo pathogen in plate assays. 16S rRNA gene sequencing identified these nine isolates as Citrobacter sp. ZA21, Bacillus spp. strains ZA33, ZA57, ZA17 and Pseudomonas spp. strains ZA20, ZA22, ZA62, ZA85 and ZA86. All the tested bacteria possessed P-solubilization and IAA production abilities. Maximum seedling vigor index observed for Citrobacter sp. ZA21 and Pseudomonas sp. ZA86 may be attributed to IAA production. Strains ZA21 and ZA86 showed effective pathogen suppression of Xoo in a pot experiment with an increase in the activity of defense related enzymes in rice plants. These bacterial strains also increased the root/ shoot length and plant weight. Root colonization by the antagonistic bacteria was observed with molecular marker such as BOX and by CLSM. During the second phase of study, previously characterized antagonistic PGP Pseudomonas sp. BRp3 capable of producing several important secondary metabolites as observed by LCMS, was used for formulation development and shelf life estimation. Different chemicals such as CMC and glycerol were tested for the development of liquid formulation. The formulated strain was evaluated in vitro and in vivo for different biocontrol and PGPR traits. Pseudomonas sp. BRp3 formulated with 2% CMC successfully retained consistency in its viable population, biocontrol activity against BLB pathogen and PGPR traits. Foliar spray applied with different concentrations of CMC (0.025, 0.05 and 0.1%) showed effective pathogen suppression. Root and phyllosphere colonization was studied using CLSM in combination with fluorescent markers. We can conclude that CMC based formulation appeared best option with regard to suppression of BLB disease.
Abstract Title:
Enhancement of Switchgrass in Degraded Soil Using Amendment and Inoculation Strategies

Primary Author Block:
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Abstract Body:
Background: Bioenergy feedstock production on degraded land has been promoted as a means for modulating land competition for food versus energy. Bioenergy feedstock can be produced on degraded land while saving the arable land for food, feed, and fiber. Major challenges are how to produce and enhance the biomass feedstock sustainably. Methods: Experiments were conducted in the greenhouse using moisture replacement microcosms (MRM) to screen strategies for enhancing biomass productivities of Panicum virgatum (switchgrass, SG) in a reclaimed surface-mined soil (RMS). Strategies included soil amendment with organic by-products (poultry litter, paper mill sludge, and vermicompost), inorganic nutrients (nitrogen and phosphorus fertilizers), or a commercial preparation of ectomycorrhizal and arbuscular mycorrhizal fungi (AMF, BioVam). Experiments were implemented with ten (10) treatments applied to enhance biomass of switchgrass (SG) on RMS. Each treatment was replicated six times. Results: After eight weeks of incubation in MRM systems, inoculation of RMS with AMF produced the highest aboveground and total biomass of 0.9g and 1.77g per microcosm container respectively. The total biomass of commercial AMF significantly (p<0.05) outperformed the control and all other treatment in the order of AMF > AMF+VC > PMS+N > VC = PMS = PL > PMS+AMF > N+P > ASL > Control. Conclusions: Our MRM findings indicate SG is most responsive to bioaugmentation strategy used in enhancing biomass feedstock production in degraded soils. The best three treatments of AMF, AMF+VC, and PMS+N is recommended to enhance biomass feedstock production on degraded land. These microcosm screening experiments allowed selection of treatments that are currently being tested in large pots for enhanced bioenergy biomass production in degraded soil.
Abstract Title:
Phytobiome: What Does Domestication Do to Beet Associated Bacteria?
Primary Author Block:
Abstract Body:
Background: Beet is a widely planted crop with its wild ancestor still growing on seashores in Europe. This gives us a unique opportunity to study influence of domestication on plant microbiome composition and its sources - soil as opposed to seeds. We hypothesized that i) beet microbiome composition would depend on plant genotype but certain organisms would be common due to their importance for the host and evolutionary history - these would be transmitted vertically via seeds, ii) different organisms would be recruited from soil depending on genotype and iii) the same organisms would be prevalent in metagenomic and culture-based analyzes. Methods: To compare microbiomes of sugar beet (cv. 'Huzar') with wild beet (B. vulgaris ssp. maritima) grown in garden soil, we amplified V3-4 fragments of 16S rRNA genes from seeds, plants and soil, converted them to Illumina libraries and sequenced the libraries on MiSeq. Bioinformatic analyzes were performed with dada2 (an R package) and Mothur, and community analysis was done with vegan and phyloseq in R. Additionally, at the end of the experiment bacteria were isolated from roots and identified by means of 16S rDNA sequencing. Results: Sugar beet and wild beet microbiomes were indeed different, however their common feature was high share of Proteobacteria. Only a small portion of organisms originated from seeds, and there was large overlap between endophytic and soil communities. Bacterial isolates turned out to represent various phyla and constituted only a small fraction of diversity recovered with metagenomics. Conclusions: In conclusion, domestication seems to have changed not only beet's genetic landscape, but also its microbiome. Soil has the largest impact on beet microbiome, however genotype modulates to some extent the spectrum of recruited organisms.
**Abstract Body:**

Introduction: Rapid urbanization and climate changes has led to fast shrinking of cultivable lands in India. Microbes adjusts their consortia to co-exist in an altered ecosystem. Mindful of this phenomenon, this research was designed to assemble a biofertilizer consortia suitable for soil reclamation in drought conditions. After extensive in vitro and in situ selection, Azospirillum ASP09 consortia were evaluated for their impact on the crop growth and production in actual drought hit fields and the data discussed.

Methodology: Field trials were carried out in a drought prone area, Tamil Nadu, India. The experimental design was completely randomized block with three replications R1, R2 and R3 in Kuruvai season. The field study contained nine treatment group comprised of different Azospirillum ASP09 consortia and a control. Oryza sativa ADT43, a hybrid rice variety was used as the test plant. Total cropping duration was 110 ± 10 days. The plant was monitored at four stages active tilling (35-40 days), panicle initiation (45-50 days), flowering (70-75 days) and harvesting (110-120 days). Plant growth and yield parameters viz., root and shoot length, dry & wet weight, leaf length, pigmentation, NPK content of the paddy, grain weight, harvest index were recorded and statistically analysed using analysis of variance (ANOVA) as applicable.

Results: Data indicate significant changes in root length and shoot length in the Azospirillum spp. consortia groups over control (p<0.01) with consistently higher root density. At harvest, maximum paddy growth and nutrient accumulation was recorded in groups comprised of Azospirillum ASP09 with or without nutrient supplement. Primary yield parameters, panicle length, number of filled/unfilled grains per panicle and grain weight reiterated the growth promoting potentials of Azospirillum ASP09 consortia with taller panicles (36.26±0.67 cms), more grains per panicle (243±7.07) and 27% increase in grain weight over control (p<0.01). NPK analysis of the grains and straw confirmed a qualitative increase in the treated group over control. Statistical analysis showed the similarity between various consortia that contained Azospirillum ASP09 in their impact on the plant yield (p>0.1). Conclusion: Biofertilizers comprising of indigenous microbial consortia with judicious nutrient amendments are critical to reclaim estranged fields for agricultural purposes.
Abstract Title:
Isolation of Nematophagous Fungi to Control Nematodes Infections in Plantain Crops

Primary Author Block:
N. M. Melendez Vazquez, J. Santos Santiago, A. G. Caro Baella, S. Malave Alicano, L. Nieves Rosa, F. Fuentes Rivera; Univ. of Puerto Rico in Humacao, Humacao, Puerto Rico

Abstract Body:
Nematodes are a group of enormous diversity, containing at least 15,000 species and many are still being discovered today. Most of them are microscopic, making them rather difficult to see with the naked eye. A handful of soil may contain thousands of nematodes, many of which are parasites to animals and plants. Particularly, some of these have been found to affect plantain crops. They affect the plant by feeding on the roots and thereby reducing the uptake of water and nutrients. Consequently, the plant will have less tolerance toward environmental stresses, such as drought and limiting nutrients deficits. Several chemicals known as nematicides are used to control nematode injuries to cultivated plants. However, these are generally toxic to humans and have deleterious effects on the environment. Hence, the purpose of this investigation is to use nematophagous fungi as an alternative bio-control mechanism against nematodes affecting agricultural crops. This project is aimed at the isolation of nematophagous fungi from soil samples obtained near the roots of injured plants. Potential nematophagous fungi are being isolated on Sabouraud Dextrose Agar and Potato Dextrose Agar. Most nematophagous fungi use specialized hyphae to trap and feed on nematodes. However, previous studies indicate those fungi do not produce trapping hyphae constitutively, but rather initiate trap-formation in response to the close presence of their prey. Hence, fungal isolates are then sub-cultured on Water Agar medium supplemented with nematodes, to test their ability to develop nematode traps. This step requires the collection of large numbers of nematodes, which are isolated from roots and soil near injured plants. Nematodes are collected using the Baermann funnel method. Captured worms are then reproduced into potato slices. At present, we have two fungal isolates, which show nematophagous activity. Our fungal isolates are both Ascomycetes, one has been preliminarily identified as Purpureocillium lilacinum while the other is still unidentified.
Session Title: AES02 - Microbiology of Agricultural Systems: Microbiology of Plants and Phytochemicals
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 3646
Poster Board Number: SATURDAY - 782

Abstract Title:
Unraveling Complexity in Multifunctional Molecular Attributes of Trichoderma Harzianum: Future Prospective of Integrated Omics Approaches

Primary Author Block:
V. Sharma1, R. Salwan1, P. N. Sharma2; 1Chandigarh Univ., Gharuan Mohali, India, 2Agricultural Univ., Palampur, India

Abstract Body:
Background: Biocontrol strains of Trichoderma are environmental friendly alternative for plant diseases management. These strains are capable of combating biotic and abiotic stresses through enhanced plant growth and nutrients uptake and boosted immune response of the associated host through adaptive transcriptome reprogramming of both partners. A wide range of biologically active repertoire including lytic enzymes, secondary metabolites of volatile and nonvolatile nature of biocontrol are involved in pathogen's exclusion through competition lytic enzymes and antibiosis. Genome-wide and proteome bases studies have helped in systematic monitoring of responsible candidates. Methods: The active culture of Trichoderma harzianum isolate ThHP-3 was grown in autoclaved mycelium of different plant pathogenic as carbon source. Protein was isolated using ammonium-sulphate precipitation and total RNA was isolated mycelium using trizol. The cDNA synthesis was performed using a mixture of oligo(dT)10-18 and random primers using verso cDNA synthesis kit. RT-qPCR was performed using SYBR-Premix ExTaqTM and expression level was estimated using 2-ΔΔCT method. Results: In current study, antagonistic T. harzianum ThHP-3 strain was evaluated for the secretion of enzymes and behavior of its different transcripts related to signal transduction, lytic enzymes, secondary metabolites and transporters against four fungal pathogens Fusarium oxysporum, Colletotrichum capsici, C. truncatum and Gloesercospora sorghi using RT-qPCR. The volatile compounds of the strain inhibited growth of pathogens. The profiling of extracellular proteins and response of its various transcripts showed differential and host specific response. The optimum expression of cyp3, abc, nrp, tga1, pmk, ech42 and glh20 varied with respect to host fungi. In addition, the optimum expression of transporters/cytochromes was also observed against F. oxysporum after 96h whereas transcripts for secondary metabolites and lytic enzymes showed significant variation against Colletotrichum spp. between 72 to 96 h. Conclusion: The current study revealed that the molecular responses of Trichoderma as biocontrol agents are complex and involve multigenes network. Further studies based on integrated translatome, metabolome and proteome will help in accurate identification of addressing the associated bottlenecks and getting better representation candidate genes to accelerate future research and realtime monitoring of candidate genes engaged in interaction with hosts.
Abstract Title:
13c-Based Tracer Analysis of Xanthomonas Oryzae Central Metabolism

Primary Author Block:
M. Shree, S. K. Masakapalli; Indian Inst. of Technology, Mandi, India

Abstract Body:
Xanthomonas oryzae (Xoo), a bacterial phytopathogen with also the ability to produce xanthan has significance in agriculture and industry. Very limited research has been done to understand the metabolism of Xanthomonas sp. and no metabolic systems biology studies involving 13C MFA have been reported on Xoo. In this study, wild type Xoo strain (BX043) was explored to understand the central metabolic pathway activities under nutrient media containing glucose, glutamate and methionine. 1H NMR analysis of the culture filtrates in triplicates has established that the cells utilise all the three available carbon sources. In order to understand the contributions of these carbon sources, we subjected the cells to a nutrient media containing 40% [U-13C6] glucose and investigated the average label incorporation in protein derived amino-acids of interest. The GC-MS based mass isotopomer analysis showed average 13C enrichment of 16 amino acids. It is inferred that BX043 mainly oxidises glucose and glutamine contributing to the activities of glycolysis, TCA cycle, entner doudoroff and pentose phosphate pathway. The results highlighted that pyruvate biosynthesis is influenced by glucose oxidation predominantly and to lower extent from glutamate via the TCA cycle and anaplerotic reactions. Absence of 13C incorporation in methionine shows the dependence of Xoo on external methionine to fulfil its metabolic necessity. In addition, only 36% tracer in histidine out of complete 40% incorporated label provided the quantitative estimates of pre-existing biomass (4%) and the oxidation of unlabelled glutamate which serves as a key prerequisite for 13C Metabolic flux analysis of Xoo. In order to establish the flux map, the Xoo cells were also fed with 60% [1-13C] glucose + 40% [U-13C6] glucose. The flux map obtained will be revealed that provides the detailed quantitative insight into Xoo metabolic phenotype in minimal media.

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Abstract Title:
In-Silico Assessment of Phytochemicals As Dengue Virus E Protein Inhibitors

Primary Author Block:
S. Malik; Punjab Univ., Lahore, Pakistan

Abstract Body:
Dengue is an infectious disease caused by dengue virus that results in Dengue shock syndrome (DSS) and Dengue haemorrhagic fever (DHF). There are four serologically distinct dengue viruses called DENV 1-4. The infectivity of dengue virus depends on the viral envelop protein E (DENV E). No specific treatment and vaccine is available for this virus because antibodies that neutralize one serotype are ineffective against another serotype and also due to continuous mutation. In this study we investigate the affinity of phytochemicals, with reported anti-viral activity against the conserve sites of all four strains of dengue E protein. DENV E proteins were docked with 36 phytochemicals and 03 drugs under development as positive control. Binding scores was used as indicator of binding efficacy. Possible conserved binding sites were determined by multiple sequence alignment. Compounds with highest binding scores were Beta-sitosterol (5976)> Rutin (5692)> Hyperoside (5358)> Rosmaric acid (4960)> Chlorogenic acid (4876)> Pterphenyl (4420)> Oleonalic acid (4375) with DENV E protein, which were all significantly higher (50 - 100%) than the used positive control ligands Fucadin, P-188 and PO2. Source of Beta-sitosterol is Scutellaria baicalensis; Rutin from Mentha piperita; Hyperoside from Pisidium guajava; whereas Rosmaric acid, Chlorogenic acid, Pterphenyl and Oleonalic acid are from Thymus vulgaris. High binding efficacy of these bioactive compounds suggests use of their source plants as herbal medicine for Dengue treatment by targeting the Dengue E protein. Keywords: Dengue virus; Dengue Envelop protein; In-silico drug designing; Phytochemicals.
Abstract Title:
Antimicrobial Effect of Partially Purified Ethyl Acetate Fraction of Methanolic, Ethanolic and Aqueous Extracts and Aqueous Fraction of Ethanolic Extract of Phyllanthus Amarus on Selected Bacterial and Fungal Isolates

Primary Author Block:
O. Adewale; Univ. of Ibadan, Ibadan, Nigeria

Abstract Body:
The use for natural products to cure diseases represents an area of great interest in which plants such as Phyllanthus amarus could be of great importance. The aim of this study is to determine the antibacterial and antifungal effects of ethyl acetate fraction of methanolic, ethanolic and aqueous extract of Phyllanthus amarus and also the aqueous fraction of ethanolic extract of Phyllanthus amarus. Ethanol, methanol and distilled water were used in the crude extraction of the active constituents of the plant. The extracts were partitioned (fractionated) to obtain a partially purified extracts with n- butane, ethyl acetate and dichloromethane (DCM). Some conventional antibiotics were also tested against the test organisms. Antibiotic disc of various antibiotics (both for Gram positive and Gram negative bacteria) were tested against the test bacteria and Ketoconazole tablet was tested against the test fungi. Agar diffusion and broth dilution methods were used for this study. Results obtained show that the fungi isolates showed some resistance to all the fractions except for the aqueous fraction. Ethanolic extract was active against 12 fungi isolates with higher zones of inhibitions than the other extracts. The highest zone recorded was 17mm and this was against Trichoderma sp. The highest zones of inhibition against bacteria isolates was 18mm and this was obtained from the ethyl acetate fraction of ethanolic extract against Escherichia coli. Overall, the fractions showed minimal or no activity against the fungi isolates particularly the ethyl acetate fraction of ethanolic, methanolic and aqueous extracts. The Minimum inhibitory concentrations (MIC) and Minimum bactericidal concentration (MBC) of the fractions on the test bacteria and fungi isolates were also carried out. The qualitative phytochemical screening of the plant showed that it contains tannin, steroid, alkaloids, and flavonoid as its bioactive constituents. If well annexed, plant extracts promise to be a viable alternative to the problem of antibiotic resistance.
Context: Salmonellae are pathogenic bacteria responsible for typhoid and paratyphoid fevers. Bacteria of the faecal peril, they cause major and minor salmonellosis, real public health problems. Market herbalists are one of the main sources of primary health care for people in developing countries. They contribute to plant conservation and endogenous knowledge. The present study aimed at establishing the potentialities of Beninese flora for the treatment of salmonellosis. Methods: It was carried out thanks to an ethnopharmacological study of medicinal plants sold. It was conducted with 90 herbalists located in 30 markets in southern Benin. The method used is the Triplet Purchase of Medicinal Recipes (ATRM). Results: During this study, 57 species of plants sold by herbalists were identified. Among these plant species, the best-selling are: Cassia siemea, Phyllantus amarus Schum & Thonn, Uvaria chamae P. Beauv., Acacia siberiana, Heterotis rotundifolia (Sm) Jacq.-Fel., Crateva adansonii DC, Citrus aurantifolia Christin and P., Acanthospermum hispidum DC, Corchorus olitorius L. and Dialium guineense Willd. Conclusions: These results form the basis of further studies aimed at experimentally evaluating the potentialities of these plants. Extracts from these plants could be a source of Enhanced Traditional Medicines (MTAs) for the treatment of salmonellosis.
Session Title: AES02 - Microbiology of Agricultural Systems: Microbiology of Plants and Phytochemicals
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 5387
Poster Board Number: SATURDAY - 787

Abstract Title:
Investigation of the Resistance-Modifying Potential of Plant Extracts against Carbapenem-Resistant Acinetobacter Baumannii

Primary Author Block:
J. Y. Park, C. L. Quave; Emory Univ., Atlanta, GA

Abstract Body:
Background: The discovery of penicillin nearly 90 years ago revolutionized the treatment of bacterial disease, drastically reducing infectious disease related deaths in both clinical and veterinary settings. However, the emergence of antibiotic resistance has seriously impeded our ability to treat bacterial infections, and new approaches are needed. Plants have a long history of use in traditional medicine for infectious disease, but the mechanistic basis of action is often poorly understood. Methods: In this study, we assessed the resistance modifying potential of plant natural products against a carbapenem-resistant isolate of Acinetobacter baumannii (FDA-CDC Antimicrobial Resistance Isolate Bank, #0035). Half of the Quave Natural Products Library (QNPL), a collection of >1,400 extracts from >500 plant species, was screened alone (256 µg/mL) and in combination with ¼ the MIC of imipenem (32 µg/mL) in an OXA-72 carbapenemase producing strain of A. baumannii. Growth inhibition testing was performed by broth microdilution method, following standard Clinical & Laboratory Standards Institute (CLSI) methods for minimum inhibitory concentration (MIC) testing. Optical density (600nm) was used to determine percent inhibition in relation to control. Results: Growth inhibition at ≥ 50% was noted in 11% of extracts tested alone. When tested in combination with imipenem, 3% of all extracts demonstrated ≥10% increase in growth inhibitory activity over treatment with either extract or antibiotic alone. Conclusions: This screening study represents an important first step in the discovery of novel chemistry for resistance modification. Plant extracts are chemically complex, representing thousands of compounds, and the active agents may only make up a small percentage of the composition. Immediate next steps include the bioassay guided fractionation of active extracts, to be followed by checkerboard assays to determine if these interactions are synergistic or additive, as based on fractional inhibitory concentration (FIC) index analysis.
Abstract Title:
Isolation and Characterization of Polycyclic Aromatic Hydrocarbon Degrading Bacteria from Effluent Water

Primary Author Block:
A. Amarakoon, F. Idroos, P. M. Manage; Univ. of Sri Jayewardenepura, Nugegoda, Sri Lanka

Abstract Body:
Poly aromatic hydrocarbons (PAHs) are lipophilic organic contaminants. Exposure to PAHs are associated with an increased risk of skin and lung cancer. PAH levels in crude edible oils vary widely and refining has no effect on higher molecular weight PAHs. Hence, effluent water released from restaurants and eateries contain PAHs in high concentrations. The present study was carried out in a restaurant site of Weras ganga park, Sri Lanka. Water and sediment samples were collected from seven locations of the sampling site and some physico-chemical parameters such as, water temperature (°C), pH, conductivity (μS/cm), Nitrate nitrogen (mg/l), total phosphate(mg/l) were measured. The PAHs that were analyzed during the study were Napthalene and Anthracene. Following the extraction of Napthalene and Anthracene from water samples, quantification was done using the HPLC-PDA. Screening for potential Napthalene and Anthracene degrading bacteria was carried out using a colorimetric assay. Potential bacterial strains that were screened by the colorimetric assay were used in degradation kinetic study using Napthalene and Anthracene separately as sole carbon sources. Degradation study confirmed two bacterial strains effective for degradation of Napthalene and Anthracene. Molecular identification of both bacteria was done 16SrRNA. The 16SrRNA analysis confirmed that Achromobacter spanius as the Napthalene degrader while, Alcaligenes faecalis as the degrader for Anthracene. A.spanius showed a mean degradation rate of 0.145 ± 0.002 ppm day-1 for Napthalene while A. faecalis degraded Anthracene at a mean rate of 0.181 ± 0.036ppm day-1. Napthalene and Anthracene degradation by A.spanius and A. faecalis was further confirmed by Fourier- transform infrared spectroscopy (FTIR) method. Optimization for Napthalene and Anthracene degradation was carried out for temperature (200C, 250C and 400C), Nitrate (0.8 mg/L,1.0 mg/L, 1.2 mg/L, 1.4 mg/L and 1.5 mg/L) and Phosphate (0.8 mg/L,1.0 mg/L, 1.2 mg/L, 1.4 mg/L and 1.5 mg/L) concentrations. Hence, A.spanius showed a maximum degradation rate of 0.156 ± 0.003 ppm day-1at 200C, 1.2 mg/L Nitrate and 1.2 mg/L Phosphate whereas A. faecalis showed a maximum degradation rate of 0.234 ± 0.014 ppm day-1at similar conditions as A.spanius. Therefore, A. spanius and A. faecalis are effective in natural bioremediation of Napthalene and Anthracene contaminated waste water released from the restaurant site of the Weras ganga park. Furthermore this is the first record on Napthalene degradation by A. spanius.
Abstract Title:
Assessment of Bioremediation Potentials of Groundnut Root and Stem on Crude Oil Contaminated Soil
Primary Author Block:
V. M. Agah1, C. C. Onochie2, O. M. Nworie3, I. R. Iroha1; 1Ebonyi State Univ., Abakaliki, Nigeria,
2Nnamdi Azikiwe Univ., Awka, Nigeria, 3Federal Univ. Ndufu Alike Ikwo, Ebonyi State, Abakaliki, Nigeria
Abstract Body:
The hazardous effect of crude oil spillage is a major challenge in the oil producing communities. In this work, contaminated soil was remediated by cultivation of Arachis hypogaeae. The study revealed the rhizoremediation abilities of Arachis hypogaeae on the crude polluted soil in which the growth rate against time in weeks was significant at SD = 2.8 ± 0.7, p<0.05. Four different groups of bacteria were isolated and identified by DNA sequence, among which was Rhizobium trifolii, Prochlorococcus marinus, Rhodopseudomonas, Escherichia coli and Bacillus pumilus. Blast results obtained revealed 93, 78-100, 72-85, 72-87, and 86% sequence identities of Rhizobium Trifolii, Prochlorococcus marinus, Rhodopseudomonas, Escherichia coli, and Bacillus pumilus, respectively. Sequenced prokaryotic organisms obtained from DNA sequencing reactions of 16SR marker were resolved into seven groups with Group III contains AMV-4_16SF_2016-04-18_DO1 and AMV-1_16SR_2016-04-18_AO5 and clustering with known prokaryotic organisms such as Rhizobium leguminosarum, Azotobacter chroococcum, Enterobacteraceae (plasmid R46), HQ 112195_Rmaldis, Azotobacter vinelandii, Rhizobium pisi and Azotobacter armeniacus. Organic compounds found in the root of Arachis hypogaeae planted on crude oil polluted soil include trimethyl-3-4 hexanedione, 2-ethyl-3-methyl-1-pentene, dimethyl-1,3,5-cycloheptatriene, 2,3-Dimethylpentane, (Z)-3-Tridecene, (Z)-2-Tridecene, n-Tetradec-1-ene, Hexadecanoic acid and n-Nonane which were mainly crude oil derivatives, while from the stem of Arachis hypogaeae planted on polluted soil include p-Dimethylbenzene, 2,4-dimethylpentane, dipropylmethane, neo-haxane, 4-methylhexanol, (Z)-2-tridecene, (Z)-4-tetradecene and Pentfluoropropionic acid. The rhizoremediation abilities of Arachis hypogaeae on the polluted soil may have served as a bulking agent with an enhanced water and minerals uptake. Key words: oil spillage, rhizoremediation, Arachis hypogaeae, Bioremediation, Abakaliki
Session Number: 249  
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Session Number: 249  
Session Type: Poster  
Session Title: AES06 - Bioremediation, Biodegradation, Biofouling and Biocorrosion I  
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Session Primary Track: Applied and Environmental Science  
Abstract Control Number: 1906  
Poster Board Number: SATURDAY - 790

Abstract Title:
Hydrocarbon Tolerance Test and Biodegradation Potential of Selected Strains of Stenotrophomonas Maltophilia Isolated from Crude Oil Contaminated Soils from Mexico

Primary Author Block:
T. O. Elufisan1, I. C. L. Rodriguez1, O. O. Oyedara2, A. Valera Sanchez1, B. O. Oluyide3, K. Mohammad1, A. D. Paz Gonzalez1, G. Rivera Sanchez1, X. Guo1, M. A. Villaloboz Lopez4; 1Centro de Biotecnologia Genomica, Inst. Politecnico Natl., Reynosa, Mexico, 2Osun state Univ. Oshogbo, Oshogbo, Nigeria, 3Ekiti state Coll. of Hlth. Sci. and Technology, Ijero Ekiti, Nigeria, 4Centro de Biotecnologia Aplicada Inst. Politecnico Natl., Tepetitla Tlaxcala Mexico, Mexico

Abstract Body:
Background: Stenotrophomonas maltophilia are known to be a very versatile group of gram-negative bacteria with different metabolic potentials among which are their potential to degrade xenobiotics, polyaromatic compounds, and organophosphates. Although Studies have shown that Stenotrophomonas maltophilia have the ability to degrade hydrocarbon, most studies have focused on the degradation of long chains alkane. In this study, we evaluated the ability of Stenotrophomonas species to tolerate and degrade Poly Aromatic Hydrocarbons. Methods: We isolated the five Stenotrophomonas maltophilia strains from Oil contaminates sites in some part of Mexico and identified them by the amplification and sequencing of the 16s rRNA region of their genome. We tested their tolerance and ability to degrade different Poly Aromatic Hydrocarbon (PAH) (Biphenyl, Phenanthrene, Phenanthridine, Naphthalene and Anthraquinone and Anthracene) by growing them in Bushnell Hass media containing one of the PAH as the only carbon source for 30days at 30o C as described by Das et al 2010. Their potentials for degrading hydrocarbon based on the emulsification of oil was evaluated as described by Dans and Chandran, 2010. The quantitative evaluation of these strains to degrade hydrocarbon was carried out on the 15th and the 30th day of the experiment using the FTIR spectrometry after the extraction of the residual hydrocarbon with hexane. Results: All isolated Stenotrophomonas maltophilia strains could tolerate 1% and 5% of 100mg/ml (= 1mg/ml and 5mg/ml) of five of the hydrocarbon tested and 1% and 5% of 40mg/ml (= 400µg/ml and 2mg/ml for Anthracene)) These showed that all strains have tendency to use hydrocarbon as carbon source at the evaluated concentration and potential to degrade them. We noted that four (ASS1, ASS2, SVIA1, SVIA2) out of the five strains have potential to emulsify oil with strain SVIA2 showing the best emulsion stability (about 72%) after 48 hours. The degradation of Naphthalene by Stenotrophomonas maltophilia isolates followed the catechol dioxygenase pathway as observed on the FTIR spectrometry. Conclusions: This study revealed the possibility of applying Stenotrophomonas maltophilia for the degradation of PAH and Bioremediation
Abstract Title:
Biodegradation of Crude Oil-Polluted Nigerian Soils by Bacterial Isolates
Primary Author Block:
C. N. Umeaku, S. E. Ezenwa, C. I. Chris-Umeaku; Chukwuemeka Odumegwu Ojukwu Univ., Uli, Nigeria
Abstract Body:
Background: Biodegradation of crude-oil polluted soil by bacterial isolates was carried out to determine the ability of the isolates to utilize and detoxify crude oil-polluted soils of Nigeria. Experimental Design: Tasks involved; sample collection from crude oil-polluted environments of Niger Delta ecological zone, physico-chemical, microbiological, adaptability tests, biochemical and molecular characterization of the isolates. Methodology: Composite samples were collected from a depth of 20cm, soil pH, moisture content, water holding capacity and total organic carbon was carried out. Serial dilution using spread plate method was used to determine the total heterotrophic bacteria and hydrocarbon utilizing bacteria. Residual total petroleum hydrocarbon was analyzed using gas chromatographic flame ionization detector method. Identity of bacterial isolates was confirmed using polymerase chain reaction techniques. Data Collection: Data was collected from results of these investigations. Analysis: Anthropogenic activities continues to cause crude oil pollution of the environment. Bacteria has the natural ability to detoxify crude oil-polluted environments. There is an urgent need to look into the ugly situation faced by the people of Niger delta ecological zone. Interpretation and Reporting of Results: pH ranged from 3.65-6.12, the decrease in pH value was as a result of the accumulation of acid metabolites. Total organic carbon content ranged from 3.4-7.1 %, this influenced many soil characteristics. Total viable counts ranged from 7.1 x 106-9x1010, the bacterial load was high for all samples, environmental indigenous bacteria were able to mineralize the crude oil in the soil. Adaptability test revealed values ranging from 0.11-0.54, all the isolates adapted well to crude oil exposure. Biochemical test results revealed Bacillus spp., Pseudomonas spp., Serratia spp., Micrococcus spp., Bacillus spp., Proteus spp., Arthrobacter spp. and Shigella spp. PCR results gave the identity of the organisms as Lysinibacillus spp. M2c, Serratia marcescens Mb4, Bacillus aerius TPM-23, Proteus mirabilis LS-3, a new isolate was encountered. TPH concentrations were in the range of 123.67 mg/l-753.32 mg/l. Conclusion: This study has established the ability of the isolates to utilize and detoxify crude oil-contaminated soils. Efforts will be made to ensure further identification and naming of the new isolate at NCBI. Serratia marcescens Mb4 and Lysinibacillus spp. M2c could be used to effectively to treat crude oil-polluted environment.
Biodegradation of Recalcitrant Melanised Chicken Feathers by Free and Immobilised Cells of Bacillus subtilis for the Production of Keratinase and Protein Hydrolysates

Primary Author Block:
I. Yusuf, M. A. Shehu, A. O. Musa, S. Yahaya; Bayero Univ. Kano, Kano, Nigeria

Abstract Body:
Background: Chicken feather wastes from slaughter and poultry houses come from different coloured chickens. The melanin-containing feather wastes are hardly used in biological processes of protease and protein productions despite their daily generation due to their tough structural nature. Methods: Free-living and gellan gum immobilised cells of Bacillus subtilis were used to degrade melanised feathers and the mixture of different coloured feathers for keratinase production and protein-rich hydrolysates

Results: The bacterium grew well and produced higher keratinase activity as well as protein hydrolysates in black feather medium compared to brown and white feather media. The bacterium was able to degrade about 90% of 5 g/l of mixed coloured feathers in 36 h. Encapsulation of the bacillus in gellan gum resulted in the increase of keratinase production from 90.9 to 117.5, 85.5 to 115.6 and 86.5 to 101.5 U/ml in black, brown and white feather media, respectively and degradation of up to 40 g/l of black and brown feathers within 60 h. Conclusions: This study demonstrated the potential use of Bacillus subtilis not only in biodegradation of highly recalcitrant melanised feathers but also in producing keratinase enzymes and valuable soluble proteins for possible industrial usage.
Abstract Title:
Identification of Novel Compounds that Affect Bacillus anthracis Germination Induction

Primary Author Block:

Abstract Body:
Background: Our goal is to develop novel decontamination strategies that can be used after an accidental or intentional release of Bacillus anthracis spores. Bacterial spores are resistant to common methods of inactivation and decontamination. However, B. anthracis spores become significantly more vulnerable to stressors such as radiation, desiccation, and antimicrobials upon germination. The resulting germinated spores are highly susceptible to secondary treatment with common disinfectants.

Methods: In this study, we performed a high-throughput screen of 30,000 compounds in order to identify compounds that enhance germination in the presence of the germinants L-alanine and inosine (AI). Using a fluorescence-based kinetic germination assay, we have identified and individually confirmed multiple compounds that increase the germination of B. anthracis spores. Results: Compared to spores in AI, spores in Compound+AI mixtures show significantly more germination after one hour. We have evaluated the effect of these compounds on alanine racemase activity and we have assessed the level of antimicrobial activity in order to learn more about the mechanism associated with these compounds. Structurally, similarities exist between compounds that seem to affect B. anthracis spore germination such as the presence of hydroxyl quinolinone carboxamides. Conclusions: Together these data suggest the feasibility of using this high-throughput screening platform to identify compounds that enhance the germination of bacterial spores, and provide insight into molecules that enhance the germination of bacterial spores. Such compounds could improve wide-area decontamination by germination induction, which minimizes hazards to personnel and the environment associated with traditional methods of spore decontamination.
Abstract Title:
Isolation & Identification of Bacillus Cereus Bacteriophage Isolated from Petroleum Product Transporting Pipelines

Primary Author Block:
A. Pedramfar, K. Beheshti Maal, S. Mirdamadian; Islamic Azad Univ. Flavarjan Branch, Flavarjan, Iran, Islamic Republic of

Abstract Body:
Background: Corrosion is the main feature of oil pipelines destruction. Microbial corrosion has been detected in various industries, especially in the oil industry due to the high frequency of corrosion damage caused by bacteria in various industries, a targeted investigation is challenging. One proposal to solve this problem is the use of bacteriophages to treat microbial corrosion. Methods: The sample pipes with the corrosion were obtained from the Gandomkar petroleum pipeline station, Iran. For screening the corrosion producing bacteria the rusted pipe samples were cultured on a selective culture medium, manganese agar. The purified individual colonies were subjected to molecular examinations. For isolating bacteria from municipal wastewater in Isfahan, spot titration and whole plate titration methods were used to isolate and detect phages in this study. Results: The cultivation of corrosion based material in manganese agar after 18 hours incubation at 30°C resulted in the isolation of cream-colored colonies that had swarming. The microscopic examinations showed spore-forming Gram-positive bacilli. By The molecular examinations, the isolated bacteria was identified as Bacillus cereus PBM - IAUF - 3 with Query ID KU145279.1 in Gene Bank. For Bacillus cereus strain PBM-IAUF-3 spot titration and whole plate titration were 5x108 PFU/ml and 5x109 PFU/ml, respectively. Phages of Bacillus cereus strain PBM-IAUF-3 was related to the Myoviridae family of phages. Conclusions: This is the first report of isolation and identification of corrosion-producing bacteria from Gandomkar petroleum pipeline station, Iran. The isolated bacterium was identified as Bacillus cereus based on molecular examinations. Also, isolated bacteriophage was identified Myoviridae by electron microscopy. This study confirmed the role of bacteria in the corrosion of oil pipelines. The biological procedures for preventing the microbial corrosion could be an asset and considered as a potential in the petroleum and industrial microbiology. Phage therapy considered as one of the economic methods for reducing the biocorrosion.
Abstract Title:
Characterization of Soil Bacterial Isolates Capable of Degrading Biodegradable Plastic Mulch Films

Primary Author Block:
J. E. Liquet y Gonzalez1, S. Bandopadhyay2, J. M. DeBruyn2;  1Univ. of Tennessee-Knoxville, Knoxville, TN, 2Univ. of Tennessee Inst. of Agriculture, Knoxville, TN

Abstract Body:
Plastic mulch films have agronomic benefits such as earlier harvest times, higher yields, reduced weeds, and improved moisture conservation. Polyethylene (PE) plastic is the most common feedstock, which poses considerable financial and environmental burdens associated with disposal after harvest. Biodegradable plastic mulch films (BDMs) are a sustainable alternative because BDMs are tilled into the soil and degraded by microbes. However, breakdown in soil can be unpredictable and the lack of research and laboratory models has precluded the understanding of the microbial degradation mechanisms. To solve this, microorganisms were extracted from soil and grown on minimal media enriched with squares of BDM films. Once pure cultures were obtained, 16S rRNA gene sequencing identified the isolates as Bacillus, Rhodococcus, and two different Streptomyces sp. The isolates were tested for degradation of two BDMs (BioAgri® and an experimental PLA/PHA film) and conventional black polyethylene plastic. All strains were capable of degrading all three plastics to some extent: BioAgri® was the most readily degraded, with the Bacillus exhibiting greatest degradation rates. Next, to study their polylactic acid (PLA) degrading capabilities we incubated the strains in PLA-coated vials and quantified the production of L-lactate, its degradation by-product. Although L-lactate levels were not significant when grown with PLA only, we observed several-fold changes in its production when glucose was added to the media. Additionally, a survey of other commonly used carbon sources indicated that the two strains had different preferences for simple carbon sources associated with PLA degradation. Taken to together, this indicates that different bacterial species are likely using different metabolic pathways and strategies in the degradation of biodegradable polymers. This work has identified and begun to characterize the degradation of BDMs by bacterial isolates, an important first step towards revealing the mechanisms of degradation in soil.
Enzymatic Degradation of Melanin by Fungal Lignin Peroxidase

Primary Author Block:
M. I. A. Ali, B. Sadaqat, N. Khatoon; Quaid-i-Azam Univ. Islamabad, Pakistan, Islamabad, Pakistan

Abstract Body:
Background: Skin darkening, a trademark of skin aging, results in accumulation of skin pigment melanin. To combat this, a wide range of skin lightening agents are commercially available, most of which inhibit melanin synthesis. These agents may have detrimental side effects and can increase the risk of skin cancer which limits their use. Decolourization of melanin can be an alternative method of skin lightening. Melanin shares structural similarity with lignin which is efficiently degraded by fungus, Phanerocheate chrysosporium with the help of enzyme lignin peroxidase suggesting that melanin could be decolorized by the fungus using the same enzyme. The current study was designed to examine the ability of Lignin peroxidase from the fungus Phanerocheate chrysosporium for decolorization of synthetic melanin.

Methods: Melanin decolourization ability of Phanerocheate chrysosporium was examined on solid and submerged media containing melanin, by visualizing the change in color of media. Veratryl aldehyde enzyme assay of submerged media was performed to confirm the presence of lignin peroxidase. Plackett-Burman design was used for enhanced production of Lignin peroxidase. Purification of lignin peroxidase was done by Ammonium precipitation and gel chromatography methods. A decolourization experiment of melanin at different environmental conditions was carried out by purified enzyme. FTIR and SEM analysis of decolorized melanin were carried out to confirm any structural changes.

Results: Phanerocheate chrysosporium decolorizes melanin with the help of Lignin peroxidase enzyme. Purified enzyme decolorized melanin effectively but the decolorization effect was more prominent in the presence of a veratryl aldehyde. The highest decolorization of 96% was observed at an enzyme concentration of 15 IU/mL in the presence of a veratryl aldehyde.

Conclusions: This study reveals that Phanerocheate chrysosporium decolorizes melanin using melanolytic enzyme lignin peroxidase. The purified enzyme decolorized melanin at conditions but the decolourization efficiency increases in the presence of veratryl aldehyde.
Abstract Title:
Microbial Production of Natural Gas from Pakistani Coal of Different Ranks
Primary Author Block:
M. I. Ali, A. Y. Malik; Quaid-i-Azam Univ. Islamabad, Pakistan, Islamabad, Pakistan
Abstract Body:
Background: Microbial solubilization of coal has been considered as a promising technology to convert raw coal into valuable products. Understanding the details of microbial coal solubilization leading up to methanogenesis, is essential in order to establish new energy production techniques and industrial processes that are cost and energy effective. The present study was aimed at investigating and exploring the prospects of possible intervention of biotechnological approaches into conventional fuel sciences for the extraction of alternative fuel options like methane. Methods: Coal samples, originating from different coal areas of Pakistan, were subjected to detailed chemical analyses. Microcosms experiments were designed to analyze the methanogenesis potential of different coal samples. Other metabolites i.e CO2, H2, CO, acetate were also observed. For this purpose, bioassay with two different exogenous microorganisms WBC2 (collected from wetlands), and IF (from PRB) were employed. Results: Among all samples, CH sample which is low volatile bituminous coal produced maximum methane with WBC2 consortium, followed by SR (subbituminous coal). Relatively lower methane level was observed with IF consortium, however, maximum concentration observed in this case was with SR coal sample. Acetate accumulated in control incubations where methanogenesis was inhibited, pointing acetoclastic pathway as a major pathway and indicated acetate utilization as well as production during the course of methanogenesis. Methanogenesis inhibited control and bioassay incubations showed nearly same levels of hydrogen, proposed that acetoclastic might be the dominant pathway for methanogenesis. Carbon dioxide and carbon monoxide was produced and consumed during the course of methane production, suggesting their role in complex methanogenic pathway chemistry. Liquid extracts were analyzed through Excitation-Emission Matrix Spectroscopy (EEMS) to obtain qualitative estimates of solubilized coal; these analyses exhibited the release of complex organic moieties. Quantitative Polymerase chain reaction analysis for mcrA functional genes suggested microbial quality as well as quantity have significant influence on methane production levels. Conclusions: On the basis of present study, a laboratory model for biotransformation of different ranked coal into methane was proposed, which gave an insight of the underlying mechanisms operative in this conversion.
Microbial Communities in Accelerated Low Water Corrosion on Marine Sheet Piling

Primary Author Block:
H. C. Phan1, S. A. Wade1, L. L. Blackall2; 1Swinburne Univ. of Technology, Hawthorn, Victoria, Australia, 2Univ. of Melbourne, Parkville, Victoria, Australia

Abstract Body:
Major infrastructure such as ports and harbours, located on the coasts throughout the world typically contain critical metal components/structures immersed in marine waters. These structures can often suffer from severe degradation at the low tide water level due to a phenomenon known as accelerated low water corrosion (ALWC), which is a form of microbiologically influenced corrosion. We obtained samples of the orange-coloured corrosion material (also known as “orange bloom”) commonly associated with ALWC from steel sheet piling at a field test site in a seaside harbour on Port Philip Bay, Victoria, Australia. These samples included a combination of corrosion by-products and microbial biofilm/biomass, which were evaluated for the presence of sulfate-reducing, acid producing and iron-related bacteria using commercial test kits. In general, positive confirmation of the presence of each of these bacterial types was found from these test kits. The microbes in the outer and inner layers of the orange bloom on different steel types, and from adjacent seawater were also studied by pure culture isolation and by metabarcoding of the V4 region of the 16S rRNA genes and analysis by the QIIME pipeline using the NCBI database as a reference. According to molecular analyses, Deltaproteobacteria (which includes many sulfate reducing bacteria) were abundant in the inner of the orange bloom compared to the outer, while sulfur oxidisers were abundant in the outer layer compared to the inner. The microbial communities varied more by the location in the orange bloom (i.e., inner or outer layer) than by the steel sheet type. Additionally, the adjacent seawater had significantly distinct microbial communities (p < 0.05) compared to the orange bloom. While more than 100 pure culture isolates were obtained from one orange bloom sample by aerobic or anaerobic incubation, there was little correlation between the results of isolation and the species identified by metabarcoding. This work provides new information on the complex microbial communities associated with ALWC and has generated an ALWC microbial culture collection which will be used in subsequent laboratory-based corrosion tests. This information will be used to assist in the development of future strategies to mitigate this major corrosion problem.
Interaction between Proteome Responses and Benzo[a]pyrene Degradation by Brevibacillus Brevis

Primary Author Block:
J. Ye, H. Qin, Y. Long; Jinan Univ., Guangzhou, China

Abstract Body:
Benzo[a]pyrene is a model compound of polycyclic aromatic hydrocarbons. The relationship between its toxicity and some target biomolecules has been investigated. To reveal a global interaction of BaP biodegradation and cellular metabolism, the transformation of 1 mg L\(^{-1}\) BaP by 0.3 g L\(^{-1}\) Brevibacillus brevis was performed in 20 mL treatment solution in the dark at 25 °C shaking on a rotary shaker at 100 r min\(^{-1}\). GC-MS analysis for benzo[a]pyrene was conducted on QP2010 equipped with a type Rxi-5MS GC column. The isobaric tags for relative and absolutely quantitation technology were used to identify cellular proteome. After treatment for 7 d, the efficiencies of benzo[a]pyrene removal, biosorption and degradation were 93%, 5% and 76%, respectively, with the production of intermediates 1-naphthol and 2-naphthol. During this process, cellular metabolism of D-galactonic acid-y-lactone, L-arginine, pyruvate methyl ester, D-xylose, D-galacturonic acid, L-asparagine, L-phenylalanine, Tween 80, L-serine, N-acetyl-D-glucosamine, \(\gamma\)-hydroxybutyric acid, L-threonine, D-glucosaminic acid, itaconic acid, glycyrl-L-glutamic acid, D-cellobiose, ketobutyric acid, phenylethylamine, D-lactose, glycerol, D-malic acid, putrescine, lactic acid, acetic acid, oxalic acid and PO43\(^{3-}\) was up-regulated. The insightful finding is that benzo[a]pyrene induced the expression of xylose isomerase for D-xylose metabolism, whereas, it inhibited \(\alpha\)-cyclodextrin metabolism. Intracellular lactic acid, acetic acid and oxalic acid at 0.1-1.2 mg L\(^{-1}\) were released stemming from their enhanced biosynthesis in the pathways of pyruvate metabolism and citrate cycle, while 5-7 mg L\(^{-1}\) of PO43- were transported for energy metabolism. After the Benzo[a]pyrene degradation, 43 proteins were significantly up-expressed for pyruvate metabolism, citrate cycle, amino acid metabolism, purine metabolism, ribosome metabolism, energy metabolism and protein synthesis.
Abstract Title:
Molecular Characterization of Lignin Modifying Enzymes in Raoultella Ornithinolytica Okoh-1
Primary Author Block:
A. O. Falade, L. V. Mabinya, U. U. Nwodo, A. I. Okoh; Univ. of Fort Hare, Alice, South Africa
Abstract Body:
Ligninolysis is topical, perhaps, due to its biotechnological significance in the valorization of lignocellulosic biomass into products of economic importance such as biofuel. Lignin modifying enzymes (LMEs) which are predominantly peroxidases and laccase are recently gaining increased attention, partly owning to their roles in ligninolysis and degradation of other recalcitrant compounds in the environment. Our preliminary study has reported the ligninolytic potential of Raoultella ornithinolytica OKOH-1. In this present study, we evaluated the bacteria for the presence of LMEs-encoding genes through specific primers in a polymerase chain reaction while the translated proteins were characterized using bioinformatics tools. Molecular analysis confirmed the presence of LME genes in R. ornithinolytica OKOH-1 with 99 % similarities to DyP type peroxidase and multicopper oxidase genes in R. ornithinolytica B6 genome. The nucleotide sequences of the detected LME genes are available in the GenBank with the respective accession numbers: MF370527 and MF374335. Furthermore, bioinformatics analysis showed that the peroxidase belongs to Class B of a DyP-type peroxidase family with a molecular weight (MW) of 17.59 kDa and isoelectric point (pI) of 4.51 while the multicopper oxidase is characterized by a MW of 17.95 kDa and pI of 10.54. The presence of DyP-type peroxidase and multicopper oxidase genes in R. ornithinolytica OKOH-1 confirms its ligninolytic potential which could be exploited for industrial applications in the energy sector.
Abstract Title:
Isolation and Molecular Characterization of Hydrocarbon Degrading Pseudomonas Aeruginosa and Staphylococcus Haemolyticus From Oil-Contaminated Soil At Arzew Oil Refinery Algeria

Primary Author Block:
F. Dilm1, K. Senouci-Rezkallah1, A. Chibani2; 1Mascara Univ., Mascara, Algeria, 2Mostaganem Univ., Mostaganem, Algeria

Abstract Body:
The spills of hydrocarbon due to the petrochemical industry are major contaminants in the environment. Bioremediation is an effective, economical and environmentally sound treatment. The purpose of our study was to isolate, screen and identify the hydrocarbon degrading bacteria from the oil-polluted soil. Two oil-contaminated soils were collected from Arzew oil refinery, North-west of Algeria. Sixteen bacterial strains were isolated using mineral salt media supplemented with 1% of crude oil; these isolates were screened for their best degradation abilities. Two selected bacterial strains designated as (P2.3 and S15.1) were identified on the basis of morphological, biochemical and molecular characterization using 16S rRNA gene sequence analysis. The sequences were compared to the closest relative species in the GenBank database of NCBI. The growth rates of the selected isolates were determined using spectrophotometer at 600 nm. Based on the partial 16S rRNA gene sequencing and phylogenetic analysis; the isolates were identified as Pseudomonas aeruginosa P2.3 and Staphylococcus haemolyticus S15. Results indicated that the isolates strains had effectively utilize crude oil as sole carbon source. Linear increase in Optical Density (OD) was observed between days 4 and 10. Pseudomonas aeruginosa P2.3 showed the highest growth in media with crude oil. This study indicates that the contaminated soil samples contain a diverse population of hydrocarbon degrading bacteria and these strains could be used for the bioremediation of oil-contaminated soil.
Nitrile Metabolism and the Amplification of Nitrilase Gene in Strains of Bacillus and Corynebacterium Isolated from Waste Leachates in Lagos, Nigeria

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Background: The wide use of nitrile compounds in different chemical industries has significantly increased the levels of nitriles in the environment. Most of the nitriles are hydrophobic, toxic compounds that are difficult to degrade. Therefore, the removal of nitrile from industrial effluents and contaminated places has become imperative. Nitrilases play a key role in the bioremediation of hazardous nitriles from environmental wastes and contaminants.

Methods: Bacterial strains capable of utilizing glutaronitrile as the sole source of carbon and nitrogen were isolated from solid waste dumpsite soils by selective enrichment culture technique. Growth was evaluated at intervals (2 days) by the intensity of turbidity (O.D 600nm) in mineral salts medium, while the metabolic products were determined using GC-FID (Hewlett Packard (HP) 5890 series II, California, USA) with an OV-3 glass column pack. Nitrilase gene was amplified by polymerase chain reaction (PCR) (94 °C for 5 min, 30 cycles consisting of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 90 s followed by a terminal incubation at 72 °C for 10 min). Results: The doubling times of Bacillus sp strain WOD8 KX774193 and Corynebacterium sp strain WOIS2 KX774194. WOD8 when grown on glutaronitrile and benzonitrile were 12.2 and 7.86 d (specific growth rate, µ: 0.057 and 0.088 d⁻¹) and 15.75 and 13.33 d (specific growth rate, µ: 0.044 and 0.052 d⁻¹) respectively. Also, strains WOD8 and WOIS2 grew on supplemented glutaronitrile and benzonitrile with doubling times of 9.76 and 7.62 d (µ: 0.071 and 0.091 d⁻¹) and 10.5 and 8.15 d (µ: 0.066 and 0.085 d⁻¹) respectively. Gas Chromatographic studies revealed that glutaronitrile degradation by the strains followed a nitrilase pathway. Based on gas chromatographic analysis, glutaronitrile residual concentrations at day 16 for strains WOD8 and WOIS2 were 35.77 gL⁻¹ (72.2 %) and 9.30 gL⁻¹ (92.5%) respectively, whereas the benzonitrile residual concentrations for the same strains were 27.39 gL⁻¹ (78.8 %) and 13.79 gL⁻¹ (89.2 %). Gene cluster for nitrilase involved in nitrile degradation was detected in the genome of the bacterial strains. Amplification of putative nitrilase gene gave polymerase chain reaction (PCR) products of sizes 1750 bp and 950 bp as expected for strains WOD8 and WOIS2 respectively. Conclusion: These novel features make the nitrilase-producing bacteria suitable candidates for in-situ application on sites contaminated with both aliphatic and aromatic nitriles.
Non Pathogenic Pseudomonas Veronii Strains for Mining Wastewater Biotreatments: Metal Sensing and Recovery Combined with Thiocyanate Degradation

Primary Author Block:

Abstract Body:
Mining processes produce extremely hazardous wastes that require efficient treatments for a safe disposal. The mining company installed in Veladero (San Juan, Argentina) uses cyanide compounds for gold leaching. These species react with the sulfide present in ores producing thiocyanate (SCN−−). Despite applied chemical treatments are directed at the removal of CN−−, SCN−− and metals, they are not ecofriendly enough to ensure non harmful discharges to the environment. The aim of this work was to study SCN−− degradation, synthesis of Au nanoparticles, Cu(II) biosensing and recovery mediated by indigenous and non pathogenic Pseudomonas veronii strains, isolated from polluted environments, for mining wastewater biotreatments. SCN−− degrader P. veronii M3 was selected and minimum inhibitory concentration (MIC) was determined in M9-glucose-0.2-40 g KSCN /L after 4 days at 20°C, 120 rpm. MIC was 7.3 g SCN−−/L, higher than the amounts found in wastes. SCN−− biodegradation was studied in M9-glucose-2.5 g KSCN/L, monitoring biomass and SCN−− removal for 71 hours. A 75 % of SCN−− decrease was registered in 65 h at 20°C. Focusing on metal recovery, extracellular biosynthesis of Au nanoparticles by P. veronii 2E was detected after 3 days at 25 °C and 120 rpm exposure of wastes to PYG culture supernatants. The nanoparticles were examined by spectroscopy for further purification. For Cu(II) recovery, metal was adsorbed from a 1 mM solution at pH = 5.5, 32 °C, using P. veronii 2E free (BL) or immobilized (BI) cells in batch reactors. An 80 -90 % of the initial Cu(II) was retained by BL, while up to 60 % Cu(II) was biosorbed by BI depending on the matrix. After optimizing Cu(II) desorption, using 0.075 M HCl (32 °C -100 rpm), an 85 % adsorbed Cu(II) was recovered from BL systems, while 76 % to 100 % Cu(II) was desorbed from BI. In this way, a 3-4 times concentrated metal extract was obtained. For Cu(II) detection in wastes an electrochemical biosensor was developed. Carbon Paste Electrode modified with P. veronii 2E (CPEM) was built using (in w/w): 30 % mineral oil; 60 % HNO3 prewashed graphite power with and 10 % 24 h-dried cells. CPEM was exposed 5 min to Cu(II) solutions (1- 50 µM, pH 5.5). After washing with ultrapure water, Cu(II) was detected by anodic stripping square wave voltammetry (0.003M HNO3), with 60 s-deposition at -1.2 V. Cu(II) peak was observed at 0.15 V. Surface could be regenerated by immersing 30 s in 1.5 M HNO3. P. veronii M3 and 2E responses allowed SCN−− removal, metal recovery and Cu(II) biosensing resulting a multiple tool for ecological mining wastewater biotreatments.
Abstract Title:
Comparative Study of Polystyrene and Polyethylene Degradation by Insect Larvae

Primary Author Block:
A. Navlekar, D. Carr; Texas Tech Univ., Lubbock, TX

Abstract Body:
In recent years, microbial plastic degradation has become a huge area of interest. Currently, plastic disposal occurs by recycling, conversion to a different product or chemical degradation. Until very recently, polyethylene (PE) required certain chemical additives to make it degradable. To find a more prudent way to deal with this challenge, researchers have concentrated their efforts on using bioremediation as a solution to the plastic disposal problem. Yang et al. (2014) showed that polyethylene was degraded by the gut microbial community of Plodia interpunctella (larvae of Indianmeal moths). While the gut microbiota of these larvae may possess plastic degrading capabilities, the process itself is unclear. The types of microorganisms involved and any changes in their abundances during PE degradation is still unknown. We have recently completed the metagenomic study of polystyrene (PS) degradation by Tenebrio molitor (larvae of the Mealworm beetle). We showed that the native gut microbial community of Tenebrio itself can degrade polystyrene without much change to its community composition or structure. However, there were certain changes seen in microbial abundances. The most abundant species found in the control were Enterococcus, Akkermansia, Bacteroidales, Lactococcus and Vagococcus, to name a few. In PS-fed Tenebrio, abundance of Enterococcus sp. was shown to have significantly decreased while that of Vagococcus increased. The current study reports on metagenomic diversity and identification of major gut microbial species present in Plodia fed with Polyethylene only, compared to Plodia fed a normal diet of bran. Microbial DNA extracted from these larvae was sequenced by targeting the v3 and v4 regions of 16S rRNA. Qiime and Usearch analysis pipelines helped identify the microbial species along with statistical tests to determine any significant changes in abundances. Using Qiime, we show that the following microbial groups significantly increased in those larvae with PE as their sole source of food: Tepidimonas, Pseudomonas, Rhizobales and Methylobacteriaceae. On the contrary, a sharp decrease in abundance of Firmicutes, specifically Turicibacter, indicated that they might not be involved in the polyethylene degradation process. Results from this study contribute to understanding of the functionality of this mixed community that enables it to degrade PE successfully. Applications for plastic recycling, one of the most intractable problems of human society today, are expected deliverables from this study.
Abstract Title:
Cold-Adapted Denitrifiers in Woodchip Bioreactors
Primary Author Block:
E. Anderson1, J. Jang1, S. Ishii1, R. T. Venterea2; 1Univ. of Minnesota, St Paul, MN, 2USDA-ARS, St Paul, MN

Abstract Body:
Background: Nitrate runoff from agricultural fields can cause eutrophication in surrounding rivers, lakes and oceans. Woodchip bioreactors are used to reduce nitrate leaching through a biological denitrification reaction. However, they do not function well in the spring when runoff and nutrient leaching are at high levels, most likely due to cold temperatures. In this study, we aimed to identify cold-adapted denitrifiers in woodchip bioreactors by using culture-dependent and -independent methods.

Methods: Woodchip samples were collected from the field bioreactors near Willmar, Minnesota, and anaerobically incubated with and without nitrate and/or acetate at 15ºC. Occurrence of denitrification was detected only when woodchips were incubated with nitrate. DNA and RNA were extracted from the woodchips at 12, 24, and 48 hours after incubation. Bacteria active under denitrification conditions (samples incubated with nitrate) were identified by comparative 16S rRNA gene sequencing analyses. In addition, denitrifying bacteria were identified in the same woodchip samples by a culture-based approach. Results: Distinct community structures were identified between woodchips incubated with and without nitrate, suggesting that addition of nitrate influenced microbial communities. Diverse genera such as Flavobacterium, Pseudomonas, and Polaromonas were identified abundantly present in the woodchip samples under the denitrification conditions by the culture-independent approach. Cellulomonas sp. and Clostridium sp. were the main denitrifiers identified by the culture-dependent method. Among those, Cellulomonas spp. was commonly detected by the two methods.

Conclusions: We identified denitrifying bacteria active in the woodchip samples under cold temperatures. By inoculating these bacteria to the field bioreactors, nitrate removal could be improved.
Abstract Title:
Biodegradation of Azo Dyes by the Microbial Consortium Cp23 Isolated from Textile Wastewater of Lima, Peru
Primary Author Block:
Abstract Body:
The textile industry pours into the environment tons of wastewater containing azo dyes, which are toxic, carcinogenic, mutagenic and negatively affect the entire life. Current azo dyes biodegradation approaches use individual strains such as Pseudomonas aeruginosa UCP 1567 that have an average yield of 89.5% after more than 100 hours. We designed and evaluated a microbial consortium for improve the biodegradative capacities at 100 ppm of azo dyes in less time. The microbial consortium CP23 was constructed, using the Enterococcus gallinarum PL23 and Stenotrophomonas maltophilia LS23 Strains isolated from textile wastewater from a factory located in Lima, Peru. Both strains were identified through 16S ribosomal RNA sequencing and a sequence similarity of 99% for both strains were determined with the Enterococcus gallinarum BM4174 and Stenotrophomonas maltophilia K279a sequences. The CP23 microbial consortium growth kinetics was evaluated through the culture in ZZ medium supplemented with 100 ppm of Direct Blue and the counting in Petroff-Hausser chamber. The biodegradation yields for 8 azo dyes were evaluated through UV-Vis spectrophotometry by 6 hours of culture. Finally, the most important parameters for in vitro biodegradation were optimized: carbon and nitrogen source concentration, culture temperature, inoculum size and initial pH. The doubling time and the growth speed (μ) were 26.5 ± 0.5 min and 2.6x10^2 ± 0.05 h-1 respectively for the CP23 consortium. The yield for the biodegradation of Direct Blue, Drimaren Yellow, Drimaren Red, Remazol Navy Blue, Remazol Blue, Remazol Golden Yellow, Remazol Turquoise Blue and Remazol Red were 97.75, 95.50, 95.60, 99.02, 98.77, 84.92, 63.14 and 97.56% respectively. The improved conditions for in vitro biodegradation were 0.5% glucose, 1% yeast extract, pH 8, 37 °C, 2 x 10^6 CFU x mL-1 of initial inoculum and 100 ppm of dye. In contrast to the largest time necessity for azo dyes biodegradation using individual strains, the CP23 consortium reached an excellent degradative capacity with an average yield of 91.53% at 6 hours. This mixed culture can be applied to wastewater decontamination systems containing azo dyes.
Abstract Title:
Oil Biodegradation in Hypoxic Waters of the Caspian Sea
Primary Author Block:
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Abstract Body:
Background: Many geochemical and hydrological studies of the Caspian Sea exist, but information on its microbial community is sparse. It is a closed basin (riverine inflow, no outflow) with large inputs of anthropogenic and natural hydrocarbons (eg, from mud volcanoes). These factors combine with hypoxia in the deep water creating a unique niche, the effects of which on the microbial community have not been investigated. Methods: Ambient water was collected at 6 sites and 4 depths for analysis and microcosm experiments. Community structure of all samples was analyzed by 16S rRNA gene amplicon sequencing. Microcosm experiments were amended with 100 ppm oil hydrocarbons or no oil (control). On ship microcosms were maintained with atmospheric headspace, and destructively sampled at 0, 24, and 72 hours. In lab microcosms were monitored for CO2 respiration under nitrogen vs atmospheric headspace and destructively sampled at 0, 3, and 17 days for sequencing and hydrocarbon analysis by GC/MS. Results: The ambient community was dominated by Gammaproteobacteria suggestive of oil-degraders. Deep sites were co-dominated by Crenarchaeota suggesting nutrient limitation or microaerophilic conditions. On ship microcosm communities differed by depth of sample origin but not by oil amendment, suggesting oil biodegradation may be influenced by environmental factors eg, oxygen concentration. In lab microcosm communities were dominated by Gammaproteobacteria, including enrichment of known hydrocarbon degraders (e.g., Oceanospirillacea) by 17 days. Oil amended microcosms respired more CO2 than controls (no oil). Oxic and anoxic microcosms degraded similar fractions of total hydrocarbons, but anoxic microcosms degraded substantially more 4 ring PAHs (100% vs 10%). The approximate half-life for total hydrocarbons was 11 days (anoxic) and 15 days (oxic). Conclusions: The half-life of the oil hydrocarbons is similar to other marine environments, but it is unexpected that anoxic loss exceeds oxic. Relative to surface waters, the microbial community in deep waters may have improved oil hydrocarbon biodegradation as an adaptation to the low oxygen, low nutrient environment.
Abstract Title:
Utilizing Comparative Metatranscriptomics to Discover Biomarkers for Anaerobic O-Xylene Biodegradation

Primary Author Block:
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Abstract Body:
Molecular biology assays are typically developed based on sequences from pure cultures, making traditional approaches time-consuming. Furthermore, the relevance of assays based on pure culture to field applications is often unknown, because the natural microbial community is poorly represented in culture. Critically, assay accuracy can be severely limited due to primer-template mismatches. Therefore, there is a critical need for new strategies to identify biomarkers that are relevant to a targeted environment. To address this challenge, we explored the relative merits of comparative metatranscriptomics and a complementary DNA (cDNA) subtraction approach for molecular assay development. We used a methanogenic o-xylene degrading enrichment culture as the model system. The model system was chosen because current assays for anaerobic o-xylene biodegradation are based on the hypothesis that the initial step in biodegradation proceeds via fumarate addition by benzylsuccinate synthase, encoded by the biomarker gene bssA. However, this is not confirmed and the pathway for anaerobic o-xylene degradation is currently unknown. To identify novel biomarkers, we extracted RNA from the methanogenic enrichment cultures under active o-xylene degradation, and under non-degrading (with glucose) conditions. Metatranscriptomes were sequenced under the different culture conditions, assembled, annotated, and differentially expressed genes were identified. In parallel, mRNA from the total RNA pool of each culture condition was enriched, and cDNA was synthesized. A subtractive hybridization was performed to enrich differentially expressed genes, based on the principle that transcripts common to both culture conditions hybridize to form double-stranded DNA, which was then selectively removed. The enriched pool of differentially expressed cDNA was also sequenced and annotated. The set of genes identified by metatranscriptomics was compared to the set identified by cDNA subtraction to determine the relative merits of each approach for identifying new biomarkers, which method is best for detecting low-abundance genes that are differentially expressed, and which method may be most applicable to field studies.
Abstract Title:
Shining Light on Anoxygenic Phototrophic Bacteria: A Syntrophy of Teamwork for Hydrocarbon Degradation

Primary Author Block:
T. C. Reid1, I. G. Droppo2, S. Chaganti1, C. G. Weisener1; 1Univ. of Windsor, Windsor, ON, Canada, 2Environment and Climate Change Canada, Burlington, ON, Canada

Abstract Body:
Background: Microbial populations in both aquatic and terrestrial environments deal with a constant flux of nutrients and xenobiotic substances both naturally and resulting from anthropogenic influences. Advancements in genetic sequencing techniques is providing great insight is into how microbes are dealing with such changes, and how microbes have and continue to alter global biogeochemical cycling. Fingerprinting these types of compromised environments has become a dominant topic of interest within the scientific community, though what often remains unstudied are natural/baseline environments, providing crucial “reference” parameters from which to compare these contaminant sites. In terms of bitumen mine reclamation, the case for understanding baseline environments is perhaps most pertinent in End-Pit Lake (EPL) reclamation research. These EPLs are a proposed reclamation strategy once mining has ceased, where extraction wastes (water, clays, sands, residual bitumen etc.) are pumped into the leftover mine pits. However, to date, little is known about the long-term fate of these proposed EPLs, and even less is known about the functional capabilities of the indigenous microbial populations, particularly their biodegradation potential.

Methods: Accessed via helicopter, sediment cores were taken from several sample sites transecting hydrocarbon-rich water reservoirs in Alberta, Canada. RNA was extracted from preserved sediment aliquots, check for QC, and sequenced on the Illumina HiSeq 2000 at Genome Quebec. Characterization of energy and xenobiotic degradation processes was performed following processing with Metatrans Pipeline and DESeq2.

Results: Differential gene expression analysis revealed significant variation between sample sites, driven by oxygen availability at the sediment-water interface. A unique syntrophy of metabolism was observed between phototrophic organisms, sulfate reducers and methanogens. Xenobiotic degradation transcripts indicate ongoing biodegradation because of natural hydrocarbon presence.

Conclusions: Observed trends in gene expression indicate a cooperative metabolism between microbial species, reliant on both oxygenic and anoxygenic photosynthesis. The complexity of the syntrophic interactions observed between microbial species provides context as to how these microbes can be so metabolically efficient at degrading compounds in a theoretically unfavorable thermodynamic environment.
Influence of Hp-β-Cd-Enhanced Solubilization and Diesel-Enhanced Catabolic Activity on Benzo[a]pyrene Biodegradation in Four Soils

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Abstract Body:
Background: The key challenges to benzo[a]pyrene biodegradation are low bioavailability and poor catabolic potentials in soils. This work evaluates the relative contributions of these factors to benzo[a]pyrene biodegradation in four soils with differing biotic and abiotic properties. Methods: The solubilizing effects of hydroxypropyl-β-cyclodextrin (HP-β-CD: 50 mM) and catabolic enhancing potential of diesel (500 and 5000 mgoil-C kg-1soil) on 14C-benzo[a]pyrene mineralisation in previously long-term contaminated soils were assessed in the soils using standard respiratory assays for 30 d. Results: The solubility-enhancement agent, hydroxypropyl-β-cyclodextrin (HP-β-CD), significantly improved the apparent aqueous dissolution of benzo[a]pyrene from soil matrices; effect decreased as soil organic matter and clay contents increased. Overall, 14C-benzo[a]pyrene mineralisation was significantly enhanced in all soils pre-exposed to diesel (0.05 and 0.5% w/w for 150 d); this been greater at higher diesel concentrations. Addition of fresh diesel (0.05%) to pre-exposed soils enhanced mineralisation further. However, the presence of HP-β-CD reduced the extents of 14C-benzo[a]pyrene mineralisation in three of the soils. Conclusion: Results indicated that the presence of catabolically-competent microorganisms and suitable co-substrates has greater effects than enhanced bioavailability to facilitate extensive benzo[a]pyrene mineralisation. HP-β-CD-enhanced solubilisation of benzo[a]pyrene without subsequent mineralisation may increase the risk of underground aquifer contamination.
Abstract Title:
Methanogenic Biodegradation Enhances Bio-Corrosion of Mild Steel

Primary Author Block:
C. C. Okoro; Federal Univ., Ndifu-Alike Ikwo, Nigeria, Abakaliki, Nigeria

Abstract Body:
Background: Bio-corrosion of crude oil storage tanks in a Nigerian oil producing facility indicates that oil degrading microorganisms and methanogens contributed to corrosion. Microbial community structure of crude oil sludge was investigated to establish the role of methanogens in bio-corrosion and also to determine if biodegradation of crude in the storage tank enhances bio-corrosion. Methods: Physiochemical analysis of samples and corrosion testing was carried out as described in Okoro et al. (2014) while biodegradation tests were carried out as described in Mills et al. (1978) and Kleikemper et al. (2005). Gas chromatographic analysis was carried out as described in Agrawal et al. (2012). DNA extraction, amplification, sequencing and bioinformatics analysis was carried out as described in Okoro et al. (2014). The entire set of raw reads is available from the sequence read archive at the National Centre for Biotechnology information (NCBI) under accession number SRR1508445. Results: 16S rRNA gene sequences recovered from crude oil storage tank samples revealed significant presence of Marinobacterium (63%), Pseudomonas (3%) alongside with acetotrophic Methanosaeta (16%) and hydrogenotrophic Methanobacterium (5%). The resident microbial community was able to reduce the gravimetric weight of residual oil by 65.5% (with complete degradation of C5-C17 nAlkane fractions) in non-amended samples and 94.13% (with complete degradation of C5-C25 nAlkane fractions) in substrate amended samples during the 60-day incubation period. Respective volume of methane produced and corrosion rates observed were higher in highly biodegraded samples (3.60mmol/0.084 mm/yr) than lesser biodegraded samples (1.64 mmol/0.018 mm/yr). Conclusion: Results showed that the resident methanogenic archaea were largely responsible for the anaerobic biodegradation of hydrocarbons in crude oil sludge and biodegradation were enhanced with substrate amendment which further accelerated the corrosion rates of mild steel coupons. Considering the relatively high number of facultatively anaerobic Marinobacterium and significant presence of Pseudomonas in the sequenced data, we speculate that the bacteria were at least partially responsible for biodegradation of crude oil components potentially acting as syntrophic organisms with methanogens to convert crude oil to methane and subsequently enhance corrosion rates of mild steel coupons.
Paint and solvents used in acrylic and oil painting generates waste that is resistant to chemical breakdown, requires expensive disposal fees, and causes health hazards during storage. Painting byproduct storage containers were found to have bacteria growing in them that could be metabolizing paint waste. Microbial degradation of three paint solvents, linseed oil, bestine, and turpenoid, by bacteria isolated from paint waste containers was investigated. In addition, bacterial strains previously isolated from jet fuel-contaminated soil were also tested for their ability to degrade these three solvents. All bacterial isolates were propagated in M9 minimal media broth containing each solvent with the majority forming biofilms at the solvent/broth interface after three weeks of incubation at 22oC. Eight of 16 isolates have been identified by 16S rRNA sequencing with taxonomic analysis of remaining isolates underway. Identified isolates from paint waste containers include Pseudomonas zhaodongensis, Planococcus citreus, and Planococcus rifletoensis. Gas chromatography mass spectrometry (GC/MS) was used to measure microbial degradation of two solvents. GC/MS results indicate six bacterial isolates degrade both bestine and oleic acid, a selected component of turpenoid, as a number of new peaks (breakdown products) were detected and the initial solvent peak areas decreased over time. Results show bacterial strains isolated from the paint waste and from jet fuel-contaminated soil have the ability to degrade individual paint waste solvents. Optimizing growth conditions (pH, oxygen, and temperature) indicates modest changes in container handling can maximize solvent biodegradation. Once the most efficient bacterial strains and their optimum growth parameters are identified, they could be inoculated into waste containers to degrade paint waste, reducing disposal fees and health risks.
Abstract Title:
Enhancing Organonitrile-Containing Wastewater Treatment Efficiency by Combining A Recombinant Bacterium with Organonitrile-Degrading and Biofilm-Forming Capability and A Positively Charged Carrier

Primary Author Block:
C. Li, Y. Sun, Z. Yue, M. Huang, J. Wang, X. Chen, X. An, H. Zang, D. Li, N. Hou; Northeast Agricultural Univ., Harbin, China

Abstract Body:
Background: Organonitriles are widely used as feedstock, and they possess carcinogenic and mutagenic characteristics. Worldwide, large quantities of organonitriles are consumed per year and discharged as wastewater. Therefore, organonitrile-containing wastewater must be treated using easier and more eco-friendly processes. The immobilization of organonitrile-degrading bacteria via the addition of biofilm-forming bacteria represents a promising technology for the treatment of organonitrile-containing wastewater, but simple mixture may reduce the biodegradation efficiency. In this study, we evaluated the effectiveness of organonitrile-containing wastewater treatment by combining a recombinant bacterium with organonitrile-degrading and biofilm-forming capability and a positively charged carrier. Methods: Nitrile hydratase and amidase genes, which play critical roles in organonitriles degradation, were cloned and transformed into the biofilm-forming bacterium Bacillus subtilis N4 to construct a recombinant bacterium B. subtilis N4/pHTnha-am. The polyethylene carrier surface was modified by anchoring ethylenediamine to obtain an amine-functionalized carrier surface, which promotes biofilm formation. Four parallel moving bed biofilm reactors (MBBR) were set up and inoculated with different strains to estimate whether the strain N4/pHTnha-am improved the efficiency of organonitriles degradation. The structure of microbial communities in biofilms through high-throughput technology was explored. Quorum-sensing genes changes among reactors were predicted by analyzing 16S rDNA marker gene sequences. Results: The immobilized N4/pHTnha-am was resistant to organonitriles loading shocks and could remove organic cyanide ion with an initial concentration of 392.6 mg/L for 24 h in a MBBR. The imputed quorum-sensing signal and the high-throughput sequencing analysis of the biofilm indicated that B. subtilis N4/pHTnha-am was successfully immobilized and became dominant. Conclusions: The successful application of the immobilized recombinant bacterium provides an innovative concept for the biodegradation of recalcitrant compounds.
Abstract Title:
Cyanide Degradation by Bacterial Isolates from Cassava Processing Effluent

Primary Author Block:
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Abstract Body:
1 High consumption rate of cassava products in Nigeria has resulted in a high number of cassava processing industries. Cassava is normally processed before consumption as a means of detoxification and preservation. The extraction of starch from the root requires large amounts of water and the residual water contains small amounts of starch, proteins and hydrocyanic acid. This effluent is discharged, untreated, into the nearby soil, streams and rivers with detrimental effects on the environment. We evaluated indigenous bacteria for their potentials for biodegradation of cyanide from industrial wastewaters. Conductivity and pH of cassava processing effluents were determined by using the conductivity meter and pH meter, respectively. Cyanide content of the effluents was determined by distillation and titrimetric methods. Bushnell-Haas medium, containing potassium cyanide were inoculated with standardised suspension of test bacteria and incubated at 30 oC for 72 h on a rotary shaker (120 rpm). Samples were taken at 24 h intervals and examined for residual cyanide. Residual cyanide was analysed by carrying out distillation for each of the cassava effluent sample distillates and Bushnell-Haas medium at 24 h intervals. To the distillates were added ten millilitres of 1.0% alcoholic sodium hydroxide and titrated against 0.02 N silver nitrate and 1.0 ml of 0.05% alcoholic dithizone indicator. The change in colour from yellow to purple indicated residual cyanide. The mean pH and conductivity of the effluent samples were 5.93 ± 0.14 and 1148 ± 232.23 µScm-1, respectively. The observed cyanide concentration in the effluents ranged from 8.43 to 14.10 mgHCN. The mean cyanide concentration was 11.56 ± 0.64 mgHCN. The three test bacterial isolates utilized completely potassium cyanide as sole source of nitrogen, in the prepared industrial cyanide (50 mg 100mL-1), after 72 h exposure. Our findings indicate that three bacterial species - Lactobacillus fermenti, Bacillus badius and Pseudomonas aeruginosa were the most effective in removal of cyanide from cassava processing effluent. They utilized completely industrial potassium cyanide as sole source of nitrogen after 72 h exposure. Hydrogen cyanide concentrations observed in the effluents were high indicating the presence of toxic levels of cyanogen glycosides in the cassava species being processed. This study indicated great potential for biological control of environmental pollution through microbial removal of cyanide from cassava processing wastewater.
Abstract Title:
Identification of Bacteria from A Soil Community Capable of Growth on A Byproduct of Ethanol Production

Primary Author Block:

Abstract Body:
Background: During commercial ethanol production a liquid syrup byproduct is made in large quantities. This study explored a possible beneficial application of the syrup by using it as a medium for bacterial growth. The long-term goal was to repurpose the resulting bacterial biomass as a protein supplement in the feeds of aquaculture grown animals. Methods: Anaerobic batch reactors were used to enrich for soil bacteria that could use the syrup as the sole nutrient source. Five samples were obtained temporally from eight-day enrichment cultures grown in triplicate to observe shifts in the bacterial community structure. Amplification of the V4 variable region of the 16S ribosomal RNA (rRNA) gene was performed using barcoded primers. The resulting PCR products were sequenced using Illumina MiSeq protocols and analyzed via the program QIIME to observe shifts in the community structure during enrichment. A sample from the last time point was plated onto nutrient-rich agar plates and individual colonies were used to obtain pure culture isolates. Enrichment isolates and other laboratory stock strains were grown in microtiter plates with the syrup substrate and absorbance was monitored for both monocultures and binary combinations. Soil enrichment isolates of interest were subsequently identified at a species level using the full 16S rRNA gene and other biomarkers. Results: The alpha-diversity calculated within each reactor decreased and then increased slightly in the last time point as the enrichments progressed, showing the succession of organisms during growth on the syrup. Community changes revealed enrichment of seven bacterial families at the final time point across the three replicates: Clostridiaceae, Alicyclobacillaceae, Ruminococcaceae, Burkholderiaceae, Bacillaceae, Veillonellaceae, and Enterobacteriaceae (in order of decreasing total percentage present). Bacillaceae were recovered at the highest frequency of pure culture isolates. Results from microtiter growth plates indicated Bacillus species, commonly used as probiotics in aquaculture, have the highest growth rate and yield of all the monocultures examined. Binary combinations of the isolates yielded no significant synergism between organisms, suggesting competition for nutrients instead of beneficial cell-cell interactions regarding metabolite conversion. Conclusion: Bacteria from pure stock cultures and from an enriched soil sample utilized the syrup as a growth substrate under facultative conditions with Bacillus species having the highest yields.
Interference of Copper Nanoparticles on the Transfer of Catabolic Plasmids by Conjugation

Primary Author Block:
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Abstract Body:
Copper nanoparticles (CuNps) are used in agriculture as a complement to pesticides due to their antimicrobial properties. CuNps generate oxidative stress and disruption of membranes on bacterial cells. The removal of pesticides is mainly done by microbial biodegradation; whose genes usually are found in catabolic plasmids mobilized among the microbial community by conjugation. The effect of CuNps on the transfer of catabolic plasmids by conjugation is still unknown. In this work, we determined if subinhibitory concentrations of CuNps interfere on the transfer of catabolic plasmids (pJP4 and pADP1) by conjugation. These plasmids contain the genes that allow the biodegradation of 2,4-dichlorophenoxyacetic acid and atrazine in Pseudomonas sp. ADP and Cupriavidus pinatubonensis JMP134, respectively. As receptors, mutant Pseudomonas putida KT2440 and Pseudomonas sp. RG8 strains resistant to rifampicin (rif) were used. Mating pairs ratio was 9:1 (recipient: donor) and incubated for 6 h at 30ºC in Luria Bertani (LB) broth, LB25%, LB50%, LB75% or mineral saline medium (MSM), in the presence of CuNps or CuSO4 at subinhibitory concentrations (0, 10, 20, 50 or 100 μg/ml). Transconjugants strains were selected in LB agar supplemented with HgCl2 (15 μg/ml) and rif (50 μg/ml), and checked for pesticide degradation and presence of plasmidial genes by PCR. Electron microscopy showed that CuNps were spherical (50-140 nm) and their chemical analysis by X-ray dispersions showed that possessed 87.2% copper and 12.7% oxygen. CuNps MIC values ranged from 200-500 μg/ml while CuSO4 MIC varied from 50-100 μg/ml. In both cases, MIC was the same as MBC indicating that both forms of copper showed biocidal properties. The subinhibitory concentrations evaluated did not modify the viable counts nor the degradative potential of the bacterial strains. Despite that the MIC of CuSO4 was lower than CuNps, CuSO4 (20 μg/ml) had no effect on frequency of conjugation (FC), while CuNps (20 μg/ml) in LB reduced FC in 90%. In MSM, the presence of CuNps at all concentrations evaluated had no effect on FC. The results suggest that CuNps negatively interfere on the transfer of catabolic plasmids by conjugation, but that effect could be related to the availability of organic matter. Also, the mechanism that generates reduction of FC could be related to an interference of CuNps than soluble ions. The decrease on the transfer of catabolic plasmids by CuNps could affect the biodegradative potential of microbial communities and decrease the removal of pesticides from the environment.
Abstract Title:
Bacillus Cereus & Lemna Minor; An Alliance against Chromium
Primary Author Block:
A. Rehman, M. Faisal; Univ. of the Punjab, Lahore, Pakistan

Abstract Body:
Background: Heavy metal contamination in soil, due to modern industrialization, has emerged as serious global concern. The non-degradable properties of heavy metals allow them to exist in environment for longer durations. Chromium is a potent heavy metal pollutant which is widely used in industrial settings including paint industry, leather tanning and metal finishing industry. Chromium is a non-essential metal for various life forms and has been reported to persist in various habitats due to excessive industrial consumption. The Chromium hexavalent ion Cr (VI) has been found to cause breathing complications like nasal irritation as well as ulceration, skin irritation, eardrum perforation which subsequently leads to lung carcinoma. By employing chromium reduction potential of microbes along with chromium scavenging ability of hydrophytes, a powerful strategy can be established to cope with increasing chromium contamination. Methods: In this study, Bacterial strains were isolated from samples collected geothermal springs of Chillas, Gilgit Baltistan, Pakistan. The metal resistance profile of isolates was determined against Cr-VI, Arsenic, Zinc and Manganese. The genomic DNA of selected microbes was extracted and sequenced. Among all isolates, Bacillus cereus TA2 and Bacillus cereus TA4 were selected owing to their ability to withstand high temperatures up to 45°C. The chromium tolerance potential of both TA2 and TA4 was estimated to be 500 µg ml⁻¹ and 600 µg ml⁻¹ K₂CrO₄, respectively. This ability to tolerate chromium at high concentrations made these strains a strong contender for plant microbe interaction with Lemna minor. Results: The association of TA2 and TA4 with Lemna minor raised levels of chromium reduction by these strains. Moreover, in Lemna minor, the contents of acid phosphatase, soluble proteins and peroxidase were found to be increased. However, the pigment content in Lemna minor was reduced. The bacterial strains enhanced chromium uptakes capabilities of Lemna minor. Conclusions: The ability of Plants and microbes to coexist in order to increases chances of survival has opened an avenue of immense research for science to exploit this potential for mankind’s welfare. This association has promising potential to deal with metal contaminated habitats and industrial settings. The microbe-assisted-phytoremediation can be efficiently utilized for the decontamination of Chromium contaminated areas.
Abstract Title:
Effects of Military Relevant Chemical Contaminants on A Reptilian Model Species Microbiome

Primary Author Block:
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Abstract Body:
Background: While mammalian microbiome-based studies have become ubiquitous in the medical literature, there have been limited gut microbiome studies focused on ecologically relevant vertebrate models like reptiles. Because of their relatively small home range, fast maturation, and high fecundity, lizards are an excellent reptilian terrestrial indicator species. For this study we used the green anole, Anolis carolinensis, as our model lizard. Anoles are commonly found on DoD installations in the southeastern US, and as a result have been used to assess toxicity of military relevant contaminants. We hypothesize that predictable changes in lizard gut microbiome composition, as a result of contaminant exposure, may serve as an easily assayed, noninvasive biomarker for a chemical exposure.

Methods: Fourteen day sub-acute exposures were conducted with 2,4,6-Trinitrotoluene (TNT) at a dose of 60 mg/kg of body weight. Anoles (n=7) were orally gavaged daily using corn oil as a carrier with controls only receiving soybean oil. Body weights and food consumption were monitored and fecal samples were collected for high-throughput 16S DNA sequencing and analytical chemistry at days 0 and 14. At the end of the study, organs were harvested for body burden data. Microbial community sequence analysis was accomplished using the QIIME pipeline.

Results: Significant changes in lizard weight loss (~6%) were observed in the TNT dosed anoles at completion of the experiment. Chemical analysis confirmed accumulation of TNT and TNT transformation products in tissue and fecal samples. PERMANOVA analysis of control and TNT bacterial fecal communities revealed significant differences at the end of the study with members of the Enterobacteriacea enriched for in the TNT dosed lizards.

Conclusions: Previously, members of the Enterobacteriacea have been shown to transform TNT as a result of nitroreductase activity. Such activity may have enriched for these organisms in the fecal microbiome. Predictable changes in lizard gut microbiome composition could offer an easily assayed, noninvasive biomarker for a specific chemical exposure providing enhanced scientific support to risk assessments on military installations.
Bacillus Sp. Isolated from Marine Environment Produces A Glycoprotein with Flocculating Potentials

Primary Author Block:
K. Okaiyeto, U. U. Nwodo, L. V. Mabinya, A. A. I. Okoh; Univ. of Fort Hare, Alice, South Africa

Abstract Body:
Background: Bioflocculants have gained considerable attention in various industries owing to their advantages over chemical flocculants that are associated with health problems. Consequently, it is highly imperative to search for a safer alternative flocculant which is harmless to humans and the environment. This present study assessed the bioflocculant production potential of a bacterial isolate from Algoa Bay, Eastern Cape Province in the South Africa. Methods: The 16S ribosomal deoxyribonucleic acids (rDNA) gene sequence analysis was used to determine the identity of the bacterial isolate. The optimum culture conditions for bioflocculant production by the bacterial isolate was investigated and the purified bioflocculant was then characterized. The flocculating potential of the purified bioflocculant was assessed against Tyhume water samples afterwards. Results: The 16S ribosomal deoxyribonucleic acids (rDNA) gene sequence analysis showed 98% sequence similarity to Bacillus licheniformis strain W7. Under these optimal conditions with inoculum size (5% v/v), maltose and NH4NO3 as carbon and nitrogen sources respectively, the maximum flocculating activity of 94.9% was attained after 72 h of cultivation. Chemical composition analyses showed that the purified MBF-W7 was a glycoproteins which predominantly composed of polysaccharides 73.7% (w/w) and protein 6.2% (w/w). Fourier transform infrared spectroscopy (FTIR) revealed the presence of hydroxyl, carboxyl and amino groups as the main functional groups identified in the bioflocculant molecules. MBF-W7 showed good turbidity removal potential (86.9%) and chemical oxygen demand (COD) reduction efficiency (75.3%) in Tyume River. Conclusions: The high flocculating rate of MBF-W7 makes it an attractive candidate to replace chemical flocculants utilized in water treatment.
Abstract Title:
Metagenomic and Physiological Analysis of Microbial Psychrotolerant Consortia Leaching of Metal Sulfides At Low Temperatures

Primary Author Block:

Abstract Body:
The bioleaching processes are limited by the availability of O2 and low temperatures (3°C to 15°C) in Andean mining zones. These parameters limit bacterial leaching operations because decrease the rate of iron oxidation by mesophilic microorganisms. This study aimed to isolate and characterize microbial psychrotolerant consortia leaching (MPCL) that can be used in industrial processes for metal recovery units at high altitude mining operation. We collected and cultured 11 samples of acidic water from the Volcan mining company (4261 m.a.s.l) located in the Cerro de Pasco city, the Huarón mine (4534 m.a.s.l) located in the district of Huayllay, and Yanamate Lake (4358 m.a.s.l) located at the southeast of Cerro de Pasco city. Then, the most efficient iron and sulfur oxidizers mixed cultures were selected through the culture in 9K modified medium and 9K base medium supplemented with sulfur 1% (w/v) respectively. The selected consortia growth kinetics were analyzed through the counting in Petroff-Hausser chamber along 432 hours, and the copper release kinetics on a synthetic sulphide (CuS) were evaluated through atomic absorption spectroscopy. Metagenomic DNA of the selected consortia were extracted. The amplicons libraries were built and sequenced using 27F and 518R primers. 16S rRNA gene analysis was done using the Mothur software package and the gene functional potential of each consortium were inferred from 16S data using PICRUSt. We obtained 6 consortia that oxidized copper sulfide 0.5%(w/v) and zinc sulfide 0.5%(w/v) at 5°C and the best doubling times were of 49.5 hours for QuF1 consortia in CuS and 60.8 hours for QuF4 consortia in ZnS. The direct application of the QuF1 consortia in CuS released 1.51 mg/L copper (II) for every 105 cells/ml. The consortia were mostly composed for the genus Acidithiobacillus sp. and only in one of them Leptospirillum ferrooxidans was present too. There was inferred significant proportions of cold-adaptative genes such as unsaturated fatty acid biosynthesis (p=0.024) and genes for ion-coupled transporters (p=0.020). In conclusion, these consortia can be applied to bioleaching operations at low temperature and the results represent the first study of molecular diversity and physiological characterization of MPCL isolated from mining areas at high altitude in Peru.
Abstract Title:
Lead Biosorption by Caulobacter Crescentus: Genetic and Quantitative Analysis

Primary Author Block:

Abstract Body:
Background: Microbial biosorption of lead is not well understood. Previous work by our group has demonstrated that microbes are able to precipitate lead in the form of Pb9(PO4)6, but the mechanism by which bacteria generate this lead phosphate is not known. We have isolated lead hyper-precipitating (hyp) strains of Caulobacter crescentus, a freshwater oligotrophic bacterium that is normally not an efficient lead precipitator, and also created several non-precipitating (nop) suppressor strains. Previous genetic analysis revealed that the hyp mutants have changes in the CC3625 cysteine synthase gene. We have proposed that hyp strains have increased cysteine synthase activity and that some of our nop mutants limit the availability of H2S substrate for the cysteine synthase enzyme. We present here a genetic analysis of RCCR62, a previously uncharacterized nop strain, as well as the initial quantitative analysis of the lead biosorption phenotype of our hyp mutants. Methods: Whole genome sequence analysis of RCCR62 was carried out followed by generalized transductions using bacteriophage Cr30. Quantification of lead biosorption was done by growing Caulobacter cultures to an OD600 = 0.5 and challenged with 0.2 mM lead nitrate. Samples were collected fifteen hours later and centrifuged to remove cells. Total lead concentration of the resulting supernatants was measured using inductively coupled plasma mass spectrometry (ICP-MS). Results: Genomic analysis of RCCR62 did not reveal mutations in any previously identified nop genes. However, RCCR62 does carry a missense mutation in CC1121 which encodes a phospho-adenylsulfate reductase enzyme. Importantly, this enzyme participates in the cellular conversion of sulfate to H2S. Transductions verified that the nop phenotype can be co-transduced with a Tn5 in the nearby CC1070 gene. Initial results show that the broth culture supernatants of hyp mutants have approximately 75-90% less lead than supernatants of wild-type and nop strains. Conclusions: The identification of CC1121 as a nop locus in RCCR62 is consistent with the hypothesis that H2S availability is important for the ability of our Caulobacter hyper-precipitation mutants to sequester lead. The quantification of total lead in culture supernatants suggests that lead removal by our hyp mutants is fairly rapid. Refinements to the protocol will be necessary to minimize loss of lead during sample preparation so that calculations of lead biosorption per cell can be done.
Abstract:
Biocorrosion, Metal Interactions, and Microbial Community Analysis of Iron-Oxidizing Bacteria from North Carolina Estuarine River Systems

Primary Author Block:
C. Garrison, E. Field, K. Price; East Carolina Univ., Greenville, NC

Abstract Body:
Iron-oxidizing bacteria (FeOB) play a large role in the biogeochemical cycle of iron in the environment, and recent advancements in culturing methods have shed new light on their role in steel colonization and corrosion (Emerson et al., 2010). Biological oxidation of iron may increase the rate of corrosion via FeOB colonizing the surfaces of steel structures (Little & Lee, 2007). Differences in steel type susceptibility to corrosion was assessed via a colonization study on two stainless steel types that vary in metal composition (304 and 316) to identify distribution and abundances of FeOB along a salinity gradient in two separate North Carolina estuarine river systems (Neuse and Pamlico Rivers). Stainless steel samples were deployed at five sites on each river along a salinity gradient for a period of six weeks to ensure adequate time for colonization, and collections were repeated for one year. A most probable number (MPN) method was used to estimate abundances of FeOB for each site. Results from seven deployments have shown that FeOB have been more abundant at higher salinities and more abundant on 316 steel type. This is notable because 316 steel type has an added component of molybdenum and is intended to be more corrosion resistant than 304. The differences in abundance on different steel types suggest that FeOB resistance to heavy metals can be variable and depends on the composition of the steel. Ongoing laboratory studies of heavy metal exposure (i.e. chromium, nickel, and molybdenum) on FeOB will reveal how these different metals affect metabolic functions of FeOB. This will help explain why colonization varies between steel types, and help us develop better corrosion resistance strategies in the future. Ongoing microbial community analyses of the environmental stainless steel samples will further reveal how the microbial community changes along the salinity gradient and between steel types. These combined results will reveal differences in susceptibility of stainless steel in aquatic environments and implications for the long-term preservation of commercial and private property in coastal environments characterized by tidally influenced estuarine systems.
Abstract Title:
Sequence Comparison of Alkm and Cata Genes of Acinetobacter Baumannii Isolates from Pasig River and Oil Sludge in the Philippines

Primary Author Block:
A. Tan, C. Hedreyda;  Natl. Inst. of Molecular Biology and Biotechnology, Quezon City, Philippines

Abstract Body:
Acinetobacter baumannii isolates from polluted Pasig River in the Philippines, were observed to exhibit significant bunker oil utilization. This research was focused on complete sequence analysis of alkane 1-monooxygenase (alkM) and catechol1,2-dehydrogenase (catA) genes from six Pasig River isolates, the oil sludge strain OS1 and a type strain A. baumannii (ATCC 19606) in order to gain insight on variation in alkM and catA gene sequences among Philippine strains and between genes from different species possessing AlkM and CatA enzyme homologues. Outputs from this research are expected to provide information for possible heterologous expression of the genes for use in oil cleanup. The alkM gene complete sequences (1,224 nt) of strain OS1 and Pasig River isolates exhibit 99.0% identity with the type strain alkM. Sequence alignment of alkM from strain OS1 with a Acinetobacter pittii showed 88.4% identity and significantly lower identity with bacteria from other groups, 53.3% for Marinobacter, 47.6% for Cornyebacterium, 47.3% for Alkanivorax, 45.8% from Legionella, 45.2% for Mycobacterium and 44.2% for Pseudomonas. This is consistent with reports that in non-Acinetobacter species, alkane 1-monooxygenase is encoded by the alkB gene that encode for homologous proteins of similar function. The predicted 407 amino acid sequences in AlkM from local Acinetobacter baumannii isolates were almost identical with only three amino acid variations from the type strain. The predicted amino acid sequences of AlkM and AlkB (from other species) exhibit low sequence identity The catA gene (921bp) from the Pasig River isolates and strain OS1 exhibit 98.9% identity with type strain A. baumannii. The catA sequences in species other than A. baumannii exhibit low sequence identity with A. baumannii. The predicted 306 amino acid sequence of CatA from Philippine A. baumannii isolates exhibit 100% sequence identity with type strain A. baumannii. Comparison of amino acid sequences of CatA protein in A. baumannii and predicted protein from other species revealed only 23.2 to 49.8% identity for non-Acinetobacter species and 92.5% for Acinetobacter radioresistens. Both alkM and catA genes in Acinetobacter baumannii are distinct from genes encoding homologous proteins present in other species. Minor sequence variation in both genes are observed among A. baumannii. The genes could be expressed in non-pathogenic hosts for direct use in enzyme production or for use in directed evolution studies.
Abstract Title:
Isolation and Genome Sequencing of Chitinolytic Bacteria from Shrimp Gut
Primary Author Block:
A. C. Y. Leung1, K-Y. Chau2, L-R. Liu2, F. C. Leung2; 1Davis Sr. High Sch., Davis, CA, 2The Univ. of Hong Kong, Hong Kong, Hong Kong
Abstract Body:
The aim of this project is to isolate and genome sequencing of Chitinolytic bacteria from shrimp gut. Chitin is the second most abundant biopolymer. It can be naturally found in the exoskeleton of arthropods as well as the cell walls of fungi and yeast. A total of 4 chitin degrading bacteria were isolated from functional screening and subsequently subjected to whole genome shot-gun sequencing and further bioinformatics analyses. Chitin degrading bacteria were isolated from gut contents of marine shrimps brought from local fresh seafood market in HK. Individual gut contents were collected, re-suspend with PBS buffer, inoculated onto chitin agar plates made from colloidal chitin extracted from shrimp shells, and serially diluted. Four isolates, SGB1, SGB2, SGA3, and SGA4 with highest chitin degrading activities were cultured and subjected to serial passage. Bacterial genome gDNA were extracted and subject to genome shot-gun sequencing using an Illumina MiSeq genome Sequencing platform. Sequencing read outputs for SGB2, SGA3, and SGA4 were filtered, quality trimmed and assembled reads resulting in 285993, 347094, 306955, and 416319 respect. Assembled contigs were submitted for BLAST analyses and resulting identification mapped 2 of the isolates as Aeromonas, one as Stenotrophomonas, and one as Diaphorobacter. KEGG analysis suggested that strain SGB1 and SGB2 proteins are from the Glyco Hydro_18 superfamily, SGA3 proteins are from the GH20_hexosaminidase superfamily and the SGA4 proteins are from the GH_18 like super family. KEGG analyses show all four isolates contain enzymes that can degrade chitin. SGA3, Stenotrophomonas maltophilia, and SGA4 Diaphorobacter polyhydroxyr ativorons are effective at degrading chitin in aerobic conditions and SGB1, Aeromonas sp, and SGB2, Aeromonas salmonicida are effective at degrading chitin in anaerobic conditions. Further testing, full genomic sequencing and subsequent analysis will enhance and analysis will help to verify more about these degrading chitin bacteria.
**Abstract Title:**
Cell-Free Ureases and Ureolytic Thaumarchaeota Play Important But Different Roles in Marine Sediment Nitrogen Cycling

**Primary Author Block:**
H. Dang1, H. Zhou2, F. Chen3, F. Azam4, N. Jiao1, Z. Zhang5, D. Wang1, M. G. Klotz6; 1Xiamen Univ., Xiamen, China, 2Dezhou Univ., Dezhou, China, 3Univ. of Maryland, Cambridge, MD, 4Univ. of California, La Jolla, CA, 5Ocean Univ. of China, Qingdao, China, 6Washington State Univ., Richland, WA

**Abstract Body:**
Urea is a major form of dissolved organic nitrogen and its hydrolysis may contribute substantially to inorganic nitrogenous nutrient availability in the ocean. In soils, two distinct sources of urea hydrolysis are usually detected: ureases that are associated with living organisms in vivo (i.e., the intracellular ureases) and cell-free ureases that are active but not associated with any living organism (i.e., the extracellular ureases). To date, little is known about the contributions by either ureolytic enzyme (i.e., intracellular versus extracellular) to the composition and dynamics of nitrogenous nutrients in the marine environment. Little is known about the identity, abundance and community structure of bacteria and archaea with ureolytic capacity as well as the environmental factors that control their respective contributions to nitrogen cycling in marine sediment environments, either. Here we report on the complement of intra- and extracellular urease activities in correlation with putative ureolytic bacterial and archaeal communities detected in sediments of the Bohai Sea, a highly anthropogenically-impacted marginal sea in the western Pacific Ocean. Detected intra- and extracellular urease activities were ubiquitous in sediments with similar potentials for hydrolysis of environmental urea. Analyses of ureC gene sequences determined from environmental DNA verified the genetic potential of sediment archaea and bacteria for urease function. Diverse sediment-specific archaeal ureC gene sequences affiliated with ammonia-oxidizing Thaumarchaeota were identified, whereas none of the bacterial ureC sequences were found to be affiliated with ammonia-oxidizing bacteria. Results of statistical analyses correlating potential urease activities, ureC sequence data and environmental parameters led us to deduce that intra- and extracellular ureases in sediments may play important but distinct roles in the nitrogen cycle of the Bohai Sea: The activity of extracellular ureases leads to ammonification of urea and thus the increase in environmental ammonium, whereas the intracellular urease activity, putatively attributed to ammonia-oxidizing Thaumarchaeota, appears to contribute to nitrification and thus the increase of environmental nitrite and nitrate by utilizing substrate ammonia obtained from urea hydrolysis. Our study reveals the importance of cell-free ureases and ureolytic Thaumarchaeota in the production of distinct forms of inorganic nitrogenous nutrients from environmental urea in marine sediments.
Abstract Title:
Outstanding Abstract Award: Genotypic Analysis of Escherichia coli Survival At Freshwater Beaches
Primary Author Block:
N. A. Rumball, S. McLellan; Univ. of Wisconsin-Milwaukee, Milwaukee, WI
Abstract Body:
The Great Lakes region experiences the highest percentage of beach closures as a result of poor water quality compared to any other region in the US, with roughly 3,000 beach closures annually. The current method used to determine these closures is the enumeration of E. coli, which is used as an indicator of fecal pollution due to its’ assumed host associated nature. However, research over the past several years provides growing evidence of E. coli’s long term persisance, and possible free-living state, in beach sand. The use of E. coli as a monitoring tool would be improved with the identification of those E. coli which are able to survive in the environment. The overall aim of this work is to identify the genotypes of freshwater beach survivors and identify factors that promote long term survival or growth. The ability for E. coli to survive at freshwater beaches was evaluated through burring microcosms containing isolates from freshwater beach sand, gull and sewage in the backshore sand of Lake Michigan in Milwaukee WI. It is hypothesized that those E. coli isolated from the beach sand will have an increased ability to survive at the beach. Experiments were conducted with sterile nutrient limited sand and with native sand containing its autochthonous community, for 14 and 6 weeks. It was observed that those E. coli in native sand microcosms had rapid population declines, while those in the sterilized sand had population increases, indicating that the surrounding biota at beaches plays a major role in E. coli’s ability to survive. Competition microcosms were also deployed containing 1:1 and 1:9 ratios of sand E. coli isolates to gull isolates to determine if E. coli from one source would out compete the other. Further insight into the results of the competition microcosms and the identification of survival genotypes will be reached through the comparison of the genomic sequences of those E. coli from the experiment inoculum to those that were collected from the end of the experiment. Insight into the genotypes of those environmental isolates is key to improving the accuracy of current monitoring methods, and may result in more accurate targeting of water quality advisory when they are needed and less swimming related illnesses.
Abstract Title:
Prevalence, Antibiotic Susceptibility Pattern and Publ. Hlth. Implication of Pathogenic Vibrio Spp. Isolated from Limpet (Scutellastra Cochlear) and Some Estuaries in Eastern Cape, South Africa

Primary Author Block:
O. E. Abioye, A. I. Okoh; Univ. of Fort Hare, Alice, South Africa

Abstract Body:
Background: Limpet (Scutellastra cochlear) is an important seafood recipe and an important member of the aquatic food chain. Thus its role as carrier of pathogenic Vibrio spp. was investigated. Methods: Limpet and water samples were collected from three estuaries in Eastern Cape, South Africa between December 2016 and November 2017. The samples were processed for the presence of Vibrio spp. with emphases on six pathogenic ones. Densities of Vibrio spp. in the samples were determined using MPN-PCR method while presumptive Vibrio spp. were isolated by direct plating of samples and aliquots of alkaline peptone water (APW) enriched samples on thiosulfate-citrate-bile salts-sucrose (TCBS) agar. The presumptive isolates were identified using PCR method. Randomly picked confirmed Vibrio spp. isolates (n = 201 in limpet and n = 159 in water samples) were subjected to antibiogram testing using 18 panels of antibiotics that are commonly recommended for treating Vibrio spp infections and used in epidemiological studies. Results: The densities of Vibrio spp. in limpet samples were more than that of water samples throughout the sampling period. About 88% of 201 and 80% of 159 presumptive isolates from limpet and water samples respectively were confirmed as Vibrio spp. The prevalences of Vibrio cholerae, Vibrio mimicus, Vibrio fluvialis, Vibrio alginolyticus, Vibrio vulnificus and Vibrio parahaemolyticus in limpet samples were 15.72%, 1.26%, 1.89%, 16.35%, 0% and 5.66% while that of water samples were 17.34%, 0%, 1.16%, 22.54%, 2.89% and 19.65%. All Vibrio spp. isolates from limpet samples and 99% from water samples were resistant to at least one of the antibiotic panel used for susceptibility testing. The Vibrio spp. isolated from limpet and water samples demonstrated multiple antibiotic phenotypes (MARP) that range between 3 and 8 in water and 3 and 14 in limpet samples. The multiple antibiotic resistant indices (MARI) in Vibrio spp. isolates from limpet range between 0.167 and 0.778 while that of water ranges between 0.167 and 0.444. Conclusions: The density results suggest that limpet is a concentrating reservoir for Vibrio spp. The presence of pathogenic Vibrio spp. that demonstrated MARP and high MARI also suggest that water resources and limpet we studied are potential sources of health risk to human in terms of vibrio-related infections. To the best of our knowledge, this is the first report linking the occurrence of pathogenic Vibrio spp. in limpet and water resources. Thus, we recommend more studies in this regards in the interest of public health.
Session Title: AES09 - Freshwater, Wastewater, Drinking Water, and Marine Microbiology: Natural Freshwater and Marine Environments
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 7075
Poster Board Number: SATURDAY - 831

Abstract Title: Metagenomic Insights Into the Mechanisms Underlying the Depth-Stratified Microbial Diversity Patterns in the Ocean’S Interior
Primary Author Block: D. Tsementzi, R. Conrad, A. Mezit, L. Rodriguez R, K. Konstantinidis; Georgia Inst. of Technology, Atlanta, Georgia
Abstract Body:
Culture-independent exploration of the oceans has identified depth-stratified microbial assemblages and revealed several genomic adaptations related to the deep ocean environment. However, the number of samples available from the deep sea remains limited, as is our understanding of the underlying mechanisms for several of the depth-specific patterns observed previously. Here, we aimed to provide new insights into these issues using deep Illumina sequencing of samples from highly stratified water masses in the Gulf of Mexico at 3 stations, from surface down to 2,100m. Comparison of taxonomic profiles revealed strong clustering of populations by depth, and not by location, even when including previously determined samples from geographically distant locations. In other words, close relatives of populations recovered from certain depths were identified along the depth profiles with decreasing genetic relatedness (measured by sequence identity) at increasing depth distance from the reference population/depth. Our analysis shows that this is a gradual as opposed to step-wise decrease in genetic relatedness, driven mostly by protein adaptation to the physicochemical properties (e.g., hydrostatic pressure) of the depths considered. Finally, comparison of functional distributions revealed shifts in gene content between surface and deep ocean communities, e.g., photosynthesis, phosphate metabolism and viral proteins were enriched in the surface samples, while aromatic compound metabolism, various peptide transporters, and transposases and integrases were found enriched in the deep water samples, in accordance with previous observations. The transposase signal appears to be tied to abundant members of the SAR324 lineage (nearly 40% of identified transposases), but a large diversity of transposases was also found outside of this group, indicating that the signal cannot be entirely explained by gene hitchhiking. Moreover, the SAR324 populations are abundant in a wide depth range, but only appear to harbor large numbers of transposases in the deepest samples, indicating a selective advantage of mobile genetic elements in deep ocean environments.
Abstract Title:
Phylogenomic Assoc. and Biosynthetic Gene Potential in A New Ascidian-Associated Antarctic Pseudovibrio Strain Tun.Psc04-5.14

Primary Author Block:
L. Bishop1, N. E. Avalon2, C. Riesenfeld1, B. J. Baker2, A. Murray1; 1Desert Res. Inst., Reno, NV, 2Univ. of South Florida, Tampa, FL

Abstract Body:
The Antarctic marine environment is a new frontier for natural product discovery, particularly in the context of host-associated diversity and microbially-produced novel compounds. A bacterium affiliated with the Pseudovibrio genus in the Alphaproteobacteria class, strain Tun.PSC04-5.14, was isolated from the tissue of an Antarctic ascidian, Synoicum adareanum. Based on phylogenetic (16S rRNA) and comparative genomic analysis (nucleic acid and amino acid identity), it is likely that this organism is a novel species in the Pseudovibrio genus. Genome comparisons with the other eighteen currently available Pseudovibrio genomes revealed that the genome is not only the largest (6.55 Mb), but also possesses low nucleotide and amino acid identity and early divergence of the 16s rRNA gene when compared to the other species in the genus. [AM1] Data mining efforts to elucidate the biosynthetic capacity of the str. Tun.PSC04-5.14 genome revealed 22 biosynthetic gene clusters (BGC), 8 of which are particularly interesting as they encode the biosynthetic machinery for predicted non-ribosomal peptides or polyketides which hold potential for biosynthesis of biologically active novel products. Reciprocal best hit BLAST analysis between the predicted open reading frames in the eight biosynthetic gene clusters and other Pseudovibrio genomes suggest that many parts of the clusters are unique to str. Tun.PSC04-5.14 and many may be results of transposition events. Seven of the BGC’s are flanked by both transposases and integrases suggesting that they were horizontally acquired. Thus the efforts to characterize this new Antarctic ascidian-associated alphaproteobacterium suggest it is likely a new bacterial species that holds significant potential in terms of natural product biosynthesis and serves as an example of the unexplored genomic potential of polar marine invertebrate microbiomes.
Abstract Title:
Ammonia-Oxidizing Archaea and Bacteria of Seagrass Thalassia Hemprichii in Coral Reef Ecosystems, South China Sea

Primary Author Block:
J. Ling, X. Lin, Y. Zhang, W. Zhou, Q. Yang, J. Dong; South China Sea Inst. of Oceanology, Chinese Academy of Sci., Guangzhou, China

Abstract Body:
Background: Seagrasses in coral reef ecosystems play important ecological roles by enhancing coral reef resilience under ocean acidification. However, seagrass primary productivity is typically constrained by limited nitrogen availability. Ammonia oxidation is an important process conducted by ammonia-oxidizing archaea (AOA) and bacteria (AOB), yet little information is available concerning the community structure and potential activity of seagrass AOA and AOB.

Methods: This study investigated the variations in the abundance, diversity and transcriptional activity of AOA and AOB at the DNA and transcript level from four sample types: the leaf, root, rhizosphere sediment and bulk sediment of seagrass Thalassia hemprichii in three coral reef ecosystems. DNA and complementary DNA (cDNA) were used to prepare clone libraries and DNA and cDNA quantitative PCR (qPCR) assays, targeting the ammonia monoxygenase-subunit (amoA) genes as biomarkers.

Results: The closest relatives of the obtained archaeal and bacterial amoA gene sequences recovered from DNA and cDNA libraries mainly originated from the marine environment. Moreover, all the obtained AOB sequences belong to the Nitrosomonadales cluster. Nearly all the AOA communities exhibited higher diversity than the AOB communities at the DNA level, but the qPCR data demonstrated that the abundances of AOB communities were higher than that of AOA communities based on both DNA and RNA transcripts. Collectively, most of the samples shared greater community composition similarity with samples from the same location rather than sample type. Furthermore, the abundance of archaeal amoA gene in rhizosphere sediments showed significant relationships with the ammonium concentration of sediments and the nitrogen content of plant tissue (leaf and root) at the DNA level (P<0.05). Conversely, no such relationships were found for the AOB communities.

Conclusions: The diversity of AOA communities was higher than that of AOB, though the abundance of AOB communities was greater than that of oxidizing AOB. This work provides new insight into the nitrogen cycle, particularly nitrification of seagrass meadows in coral reef ecosystems.
Activity and Molecular Study of Nitrite-Oxidizing Bacterial Communities in Oxic Seawater of the Northern South China Sea

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Nitrite-oxidizing bacteria (NOB) are chemolithoautotrophic microbes that carry out the second step of nitrification, oxidizing NO2- to NO3-. So far, most marine NOB studies focused on oxygen minimum zones (OMZs). However, most ocean waters are normoxic and the ocean's interior contains abundant nitrate, likely a result of nitrite oxidation by in situ NOB. In oxic seawater, chemolithoautotrophic CO2 fixation is mainly carried out by ammonia-oxidizing microbes and NOB. New evidence suggests the importance of NOB in CO2 fixation in non-OMZ waters (Pachiadaki et al., 2017). The South China Sea (SCS) is generally oligotrophic and oxic. Its seawater dark CO2 fixation rates are very high (Zhou et al., 2017). We hypothesize that NOB may be important players for nitrate production and CO2 fixation in SCS. Here we report the NOB activity and diversity in the northern SCS (nSCS). A research cruise was taken in June, 2015. Seawater ammonia- and nitrite-oxidation rates were measured via 15N tracer method using in situ temperature incubations. We found nitrite oxidation rates to be generally higher than ammonia oxidation rates, indicating ammonia oxidation is likely to be the rate-limiting step of nitrification in most nSCS seawater. Clone library and qPCR quantification analyses targeting key genes and transcripts of NOB, nxrB (encoding nitrite oxidoreductase subunit beta) and aclA (encoding ATP-citrate lyase large subunit), showed that bacteria affiliated with the Nitrospinaceae lineage are the major and active NOB in the nSCS. In addition, nxrB and aclA gene and transcript abundances are positively correlated (p < 0.05) with measured nitrite oxidation rates, indicating that Nitrospinaceae NOB are active for both in situ nitrite oxidation and CO2 fixation. However, the active CO2-fixing NOB may be just a subset of the bulk Nitrospinaceae NOB community in most parts of the nSCS, based on community clustering and PCoA statistical results using the obtained gene and transcript data. This further indicates that NOB activity in most nSCS water bodies may be substrate-limited, likely by the relatively low ammonia-oxidation rates. The transcript abundance ratio of aclA/nxrB is generally lower than the gene abundance ratio of aclA/nxrB, suggesting that the energy conserved from nitrite oxidation was mainly used by most NOB for maintenance metabolism rather than for growth. The different conclusions drawn from our research and from Pachiadaki et al. (2017) indicate that the marine NOB research is still challenging and more in-depth and systematic studies are needed.
Virulent Vibrio Species and Microbial Communities of the San Diego Coast

Primary Author Block:
R. E. Diner, A. E. Allen; Univ. of California, San Diego, La Jolla, CA

Abstract Body:
Members of the bacterial genus Vibrio are endemic to the marine environment, and several species can cause disease in humans. Thousands of cases of Cholera (caused by V. cholerae) and Vibriosis (caused by other species, notably V. vulnificus and V. parahaemolyticus) are reported annually worldwide with increased infections predicted as ocean temperatures rise. In San Diego County coastal water temperature and salinity are well within the range reported for potentially dangerous Vibrio species, however, despite the high exposure risk and multiple reported infections little is known about Vibrios in this region. Using digital droplet PCR we found that Vibrio species known to cause disease species were abundant at all study sites, particularly during the warm summer months. At multiple sites we detected V. vulnificus possessing virulence genes, which have been known to cause necrotizing infections with an extremely high (up to 50%) mortality rate. Some strains exhibited a remarkably high salinity tolerance, potentially enabled by the high water temperatures. 16S/18S tag sequencing revealed that although Vibrio bacteria were at times abundant, they represented a minority component of the complex microbial community. Vibrio abundance coincided with a diatom closely related to the chitin-producing Thalassiosira pseudonana, which may represent an understudied vector as Vibrios are known to attach to, metabolize, and become competent in response to chitin in the environment. This research elucidates aspects of Vibrio ecology that may help to protect human health in the marine environment.
Abstract Title: Isolation and Phenotypic Characterization of Salmonella Spp. from A Shallow Endorheic Lake in Mexico

Primary Author Block: O. Díaz-Torres, Y. Lugo-Melchor, J. de Anda-Sánchez; CIATEJ, A.C., Guadalajara, Mexico

Abstract Body:
Background: Lake Zapotlan is a shallow, subtropical and endorheic freshwater located in southern region of Jalisco State, Mexico, and is internationally recognized as a RAMSAR site. Rainy and fluvial sources are the main sources of water to the lake, which has preferably for agricultural use. It receives point source pollution from partially treated sewage from two surrounding cities, as well as non-point sources, including urban runoff, agricultural runoff and consequent deposition of sediment as a result of microbiological contamination by pathogens such as Salmonella spp. which represents one of the leading causes of intestinal illness all over the world furthermore the etiological agent of more severe systemic diseases such as typhoid and paratyphoid fevers. While water is known to be a common vehicle for the transmission of typhoidal Salmonella serovars, non-typhoidal salmonellae are mainly known as foodborne pathogens. The purpose of the study was to determine the presence and phenotypic diversity of Salmonella spp. isolated from lake Zapotlan, and in the main bodies of water that discharge in it.

Methods: A total of 63 water samples from lake Zapotlan were evaluated for the presence of Salmonella spp. by microbiological techniques for isolation, confirmation by molecular biology as PCR, and phenotypic characterization by the Kauffman-White scheme. Additionally, its association with environmental and physicochemical parameters, were determined using one-way ANOVA and PCA.

Results: Salmonella spp. were isolated from 19 (30.75%) samples. Three serotypes and one serogroup of Salmonella were identified; the most frequently isolated serotypes were S. Agona (68.42%), S. Weltevreden (5.26%), S. Typhimurium (5.26%) and the serogroup S. B. (21.05%). Salmonella spp. shows a positive correlation with the environmental and physicochemical factors such as precipitation, relative humidity, environmental temperature, turbidity and dissolved oxygen, but the prevalence of Salmonella spp. was best explained by environmental parameters.

Conclusions: The results of the presence of Salmonella spp. and its relationship with environmental and physicochemical parameters in lake Zapotlán, can be used to understand Salmonella spp. ecology in order to provide information for developing appropriate and effective strategies to prevent diseases and contamination of food produced in the basin, where water is used for agricultural irrigation.
Abstract Title:
Bacteria Associated with Planktonic Copepods Found in Lake Taal, Philippines and their Ability to Express Chitinase Activity

Primary Author Block:
C. Vicera; Univ. of Santo Tomas, Metro Manila, Philippines

Abstract Body:
Background: Chitin is the second most abundant homopolymer in nature. This can be found on crustaceans, such as the copepods, being the main structural component of their exoskeleton. Due to this, attachment of chitin-degrading bacteria on them and on their carcass is known to be one of the most important phenomena in the initiation of chitin biodegradation. Decomposition of these carcasses can be very important in nutrient regeneration wherein it can be an organic source for bacteria and other organisms in the water, driving elemental cycling, and microbial production. Few studies are done on the association of bacteria with copepods especially in tropical lakes, which is why chitin-degraders associated with it in this environment were studied.

Methodology: Copepods were collected in Lake Taal, Philippines. They were brought back to the laboratory and were processed. Morphologically different bacterial colonies were isolated and purified. They were then tested for chitin degradation, which was assessed through measurement of size of the clearing zone on colloidal chitin agar plate. Those that tested positive were identified molecularly through 16S rRNA gene sequence analysis and further tested for the effects of different environmental conditions (pH, temperature, salinity, and nutrients) on their chitinase activity. Results: A total of 81 bacteria were purified. 37 were from the copepod group Cyclopoida and 44 were from the group Calanoida. 6 isolates from the Cyclopoida and 4 from the Calanoida group showed chitin degrading activity and they were identified belonging to the genus Aeromonas (A. hydrophyla, A. veronii, and A. sobria). When tested, no chitinase activity was observed at pH 5, but an increase in chitinase activity was seen as the pH increased from 6 to 9. In terms of temperature, chitinase activity also increased with increasing temperature, i.e. 26oC, 28oC, and 30oC. Increase in salinity by addition of sodium chloride from 0.85 g/L to 3.5 g/L lead to the loss of chitinase activity. Addition of organic peptone in the media increased the chitinase activity but inorganic nitrogen such as sodium nitrate had little or no effect on it.

Conclusion: This study has shown the dominance of Aeromonas among the cultivable chitinase-producers found on planktonic copepods. Their chitinase activity was enhanced with an increase in pH, an increase in temperature, and addition of an organic nitrogen source. This study, the association between Aeromonas and planktonic copepods in tropical volcanic lake, might be the first report of such in the country.
Abstract Title:
The Impact of Riverine Infiltration on Groundwater Aquifer Microbial Communities

Primary Author Block:
N. J. Gayner, M. J. Salo, T. Grundl, R. J. Newton; Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract Body:
Shallow groundwater aquifers are an important agricultural, industrial and domestic drinking water source, and in the U.S. they account for 25% of all freshwater used. However, given their close connection to the surface, shallow groundwater aquifers are often altered by anthropogenic activities in the watershed. Microorganisms play a key role in groundwater biogeochemical reactions influencing water quality and the treatment processes needed to maintain potable water, but relatively little is known about how connections to surface water influence system biogeochemical stasis. In this study, three aquifer wells were examined within the same groundwater system located near the Fox River in Waukesha, WI. A significant portion of the Fox River’s flow comes from upstream wastewater treatment plant (WWTP) effluent. Our previous research indicated two wells are infiltrated by river water while the third well, approximately 1 mile away from the Fox River, is, seemingly, not infiltrated. There is no clear understanding of how or if river water infiltration will alter the microbial community, protein synthesis potential, and/or biogeochemistry of these drinking water wells. To address these questions, during the summer of 2017, water samples from the two contaminated wells and one pristine well were sampled on eight occasions. 2-3 L of water were filtered through a series of inline filters with decreasing pore-sizes (3 µm, 0.2 µm, and 0.1 µm). A preliminary analysis of a subset of samples from the 0.2 µm and 0.1 µm filters from all three wells using DAPI fluorescent stain and microscopy suggests microbial concentrations around 10^5 cells per mL of water in all three wells. 16S rRNA gene sequencing (illumina Mi-Seq) indicated the bacterial community composition of the wells differ greatly from those present in the WWTP effluent and Fox River and consist predominantly of unclassified taxa and bacterial guilds typical of groundwater, such as denitrifiers, iron-oxidizers, and sulfide-oxidizers. 16S rRNA gene sequencing on simultaneously extracted DNA and RNA from free-living groundwater microorganisms was used to characterize the microbial communities and protein synthesis potential of the communities (ribosomal RNA:DNA ratios) to identify patterns related to river water infiltration.
Abstract Title:
Can Elevated Salinity Trigger the Viable But Nonculturable State in the Marine Pathogen Vibrio Vulnificus?

Primary Author Block:
B. McHenry, G. Barbarite, P. J. McCarthy; FAU Harbor Branch Oceanographic Inst., Fort Pierce, FL

Abstract Body:
Vibrio bacteria are responsible for 80,000 illnesses in the United States every year, the majority of which occur in Florida. One species, Vibrio vulnificus, can cause potentially fatal wound infections in a select group of the population. These bacteria are found worldwide in estuarine environments but are never recovered on beaches or in oceanic waters. A field study was conducted to test the abundance of V. vulnificus along a salinity gradient from inshore to offshore. This research showed that these pathogens exhibit a strong negative correlation with salinity, rarely being found in areas approaching 35 ppt. To further investigate this trend, a lab study was conducted to monitor the culturability of V. vulnificus cells exposed to an elevated, sub-optimal salinity. Cells were grown in Heart Infusion broth overnight and washed twice to remove excess nutrients. Stocks were then inoculated into a control microcosm (11 ppt salinity) and a treatment microcosm (35 ppt salinity) at a density of 106 cells/mL. The number of culturable V. vulnificus was determined for both the control and treatment microcosms by plating onto CHROMagar Vibrio followed by overnight incubation at 37°C. The control microcosm maintained an average of 1.57 x 106 culturable cells/mL throughout the trial, while the treatment culture began to decline by day 1; no cells could be recovered at elevated salinity after day 7. These data suggests that elevated, oceanic salinity can significantly reduce the culturability of Vibrio vulnificus in as little as 7 days. These results may also imply the induction of the Viable But Nonculturable (VBNC) state of V. vulnificus in response to sub-optimal salinity. The VBNC state is an environmental stress response which has been studied most frequently for V. vulnificus at sub-optimal temperatures. While V. vulnificus has been shown to enter the VBNC state in response to decreased temperatures, this is the first study to indicate that this pathogen may be able to elicit the same response when exposed to elevated salinity. Understanding the tolerance range of this bacterium as well as its responses to various environmental stressors is crucial to informing the public and health care providers about this potentially fatal human pathogen.
Abstract Title:
Spatial Structure of the Upper Sediment Microbiome of An Industrialized Appalachian River, West Virginia

Primary Author Block:

Abstract Body:
The sediment microbiome is critical for riverine ecosystem processes and has extremely high diversity. However, surprisingly little is known about the composition, diversity, and distribution of riverine sediment microbiomes. Potential drivers of geographic patterns include surface water flow, local geochemistry, land-use practices, and pollutants. The relative contributions of these forces to geographic patterns and bioremediation potential are not known. The Kanawha River is a tributary of the Ohio River and serves the Charleston (WV) metropolitan area. The watershed encompasses 12,000 sq. miles and includes surface mining, acid mine drainage, logging, municipal inputs and an eighty-year chemical industry. The objective of the study was to deeply sample microbial diversity through a heavily impacted region of the river, to determine whether spatial variation is present, and to test which of the potential environmental drivers most affects spatial diversity. Sediment samples were collected from six locations along a 60 km region of the river. Two upper layers of sediment (1-5 and 6-10 cm) were collected. Illumina sequencing using Earth Microbiome Project protocols was used to target 16S rRNA gene community diversity. Chemical analysis was done with ICP-OES and Dionex Ion Chromatography. 15 million sequences were obtained from 56 sediment samples, resulting in 3.1 million paired-end reads. Beta diversity analysis (Bray-Curtis and UniFrac) showed that bacterial and archaeal OTU diversity showed spatial differentiation among locations for both layers. Abundance of major phyla was most strongly correlated with total organic carbon, sulfate, K, Mg, Mn, and Al. Spatial structure of sediment diversity was found to be most closely associated with local geochemistry; even the upper layer displayed geographic differentiation in spite of the high-volume and high sediment load of the river.
Influence of Climatic Variables on the Population of Pathogenic Marine Bacteria Isolated from Coast Waters of Indian Ocean in Dar Es Salaam, Tanzania

Primary Author Block:
J. Mollel1, P. Masimba1, R. Nondo1, S. Moyo1, J. Manyahi1, C. Lugomela2, R. Maghembe3, S. Abood1, D. Oluwayelu4; 1Muhimbili Univ. of Hlth.and Allied Sceinces, Dar es Salaam, Tanzania, United Republic of, 2Univ. of Dar es Salaam, Dar es Salaam, Tanzania, United Republic of, 3Marian Univ. Coll., Bagamoyo., Tanzania, United Republic of, 4Univ. of Ibadan, Oyo State, Nigeria

Abstract Body:
The population of marine bacteria is influenced by different factors such as the availability of nutrients, salinity, pH, plankton biomass and climatic conditions including temperature and rainfall. Some marine bacteria are associated with several diseases of public health significance, particularly cholera, which remains a major health concern in Tanzania. A total of 141 water samples were collected over a period of six months: 77 samples during the rainy season (March to May) and 64 samples during the dry season (July to September). Sampling locations included two points on the coast of the Indian Ocean in Dar es Salaam, Tanzania, representing the location with high human activity (HHA) and low human activity (LHA). Crystal VC rapid detection test (RDT) for Vibrio cholerae was performed on 127 samples while for isolation of V. cholerae, all 141 samples were cultured in TCBS agar. Thereafter, yellow colonies were sub-cultured on nutrient agar (37°C, 24 hours), and assayed for oxidase activity. Identification of bacterial isolates was performed using API 20E test on all oxidase positive isolates. Environmental data encompassing rainfall and temperature data for the sampling period were obtained from the Tanzania Meteorological Agency. The results of Crystal VC RDT revealed a low prevalence (1.6%, 2/127) of V. cholerae. The positive samples were both collected during the dry season from the location with HHA. Of the 141 samples cultured on TCBS, yellow colonies (sucrose fermenters) were observed for 123 (87.2%) samples. Of the 123 samples, 39.0% (n=48) were oxidase positive and were further analyzed using the API 20E test. Six bacterial species were identified including Vibrio fluvialis 25% (12/48), Vibrio alginolyticus 8.3% (4/48) and Pasteurella multocida 4.2% (2/48). Other bacteria were found at a lower frequency 2.1% (1/48) including Brucella species, Burkholderia cepacia, and Escherichia coli. The population of sucrose fermenting bacteria was slightly higher at the location with HHA (67/71, 94.4%) than at the location with LHA (56/70, 80%) while the population of oxidase-positive bacteria was relatively higher during the dry season (36/51, 70.6%) than the rainy season (9/72, 12.5%). Average temperature (27°C) was similar between the rainy and dry seasons. Our findings demonstrate that the coast waters of the Indian Ocean in Dar es Salaam, Tanzania harbor pathogenic bacteria. Observed trends support the findings that, the population of bacteria in the coast waters is influenced by human activities and climatic parameters such as rainfall.
Session Title: AES09 - Freshwater, Wastewater, Drinking Water, and Marine Microbiology: Natural Freshwater and Marine Environments
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 7249
Poster Board Number: SATURDAY - 842

Abstract Title:
Sampling the “Rare Biosphere” in the Sediment of A Highly Disturbed Appalachian River, West Virginia: High Diversity and Novelty
Primary Author Block:
Abstract Body:
Rivers provide essential natural resources for many municipal, industrial and agricultural processes. However, heavy use can cause severe disturbances to freshwater environments that alters biodiversity and ecosystem services. Our long-term research seeks to understand the impact of land-use practices on riverine ecosystems at a range of scales. The Kanawha River (WV) watershed, encompassing 12,000 square miles, is our model system. The river runs through the Charleston metropolitan area and has been severely impacted for eighty years; during peak industrialization regions of the river were anoxic. Whether long-term industrial disturbances decrease microbial diversity and functions is not well known for rivers. Our initial analysis has focused on sampling sediment microbial diversity at six locations along a 60 km region. Replicate samples of the top 10 cm of sediment were collected and bacterial and archaeal diversity was assessed with Illumina sequencing of 16S rRNA genes following the Earth Microbiome Project protocols. Coordinated chemical characterization of sediment was performed using Inductively Coupled Plasma Spectroscopy and Ion Chromatography. More than 15 million sequences were obtained, resulting in 3 million paired-end reads. Excluding singletons, nearly 68,000 Operational Taxonomic Units (OTUs) were found. 77 putative microbial phyla were identified; 47 are uncharacterized. 99.8% of the OTUs occurred at an abundance of <0.001%. These rare OTUs constituted 76.7% of the total sequences. About 60% of classes and orders are unnamed; 4% of the sequences had no known affiliation. The most abundant phyla were Proteobacteria (32%), Chloroflexi (11%), Bacteroidetes (8%), Acidobacteria (8%), and Plactomycetes (4%). Kanawha River sediment microbial diversity was found to be among the highest measured for any environment. The high number of uncharacterized higher level taxa and large fraction of rare OTUs are especially noteworthy.
Abstract Title:
Sediment Nutrient Loading Results to the Increase in the Density of Bacillus Spp. and Aeromonas Veronii in Sediments of Aquaculture Sites in Lake Taal, Batangas, Philippines

Primary Author Block:
J. R. Tuazon, M. A. Santos; Univ. of Santo Tomas, Manila, Philippines

Abstract Body:
Unconsumed fish-feeds utilized in the production of fish meat may end up in the sediments to accumulate, making these nutrient-rich sediments serve as ideal environment for the enrichment of microorganisms. Therefore, the density of fish-feed fermentative bacteria (FFB) from the sediments of aquaculture sites and non-aquaculture sites in Lake Taal in the Philippines was compared and studied. Sediment samples were collected from non-aquaculture sites in Cuenca, Tanauan and near the lake outlet and from aquaculture sites in San Nicolas, Agoncillo and Talisay. To determine if there was an observable trend in the density of FFB depending on the seasonal variation, samples were collected on March 2016, July 2016, October 2016 and March 2017. The density of FFB in each sampling site was determined by employing the three-tube MPN method with phenol red amended with 1% fish-feed extract as medium. Bacteria from positive terminal tubes were isolated, purified and subsequently characterized through biochemical tests and 16S rRNA sequence analysis. MPN of FFB indicates that there is usually an order of magnitude difference in the density of FFB between non-aquaculture sites and aquaculture sites (p=0.014). As were observed from the results of the MPN, density of FFB in the sediment of non-aquaculture sites were usually 103-105 MPN per milliliter of wet sediment compared to aquaculture sites which ranged from 104-107 MPN per milliliter of wet sediment. Density of FFB during seasonal variation showed that FFB were higher during dry season than wet season (p=0.016). Molecular identification showed that sediments from non-aquaculture sites were dominated by Aeromonas veronii, Bacillus spp., Paenibacillus sp. and Vibrio cholerae. While aquaculture sites were dominated by Aeromonas veronii and Bacillus spp. Findings indicated that the presence of aquaculture activity in an area in the lake could result to an increase in the density of FFB in the sediment.
Abstract Title:
Exploring the Marine Microbial Food Web and Viral Shunt Using Patterns of Diversity and Gene Expression Within the California Current Long-Term Ecosystem Observatory

Primary Author Block:
L. Zeigler Allen1, A. J. Rabines2, J. P. McCrow1, H. Zheng1, K. Goodwin3, M. Bohan4, A. E. Allen1; 1J Craig Venter Inst., La Jolla, CA, 2Univ. of California, San Diego, La Jolla, CA, 3NOAA, Miami, FL, 4NOAA, Silver Spring, MD

Abstract Body:
Quarterly collection of samples suitable for DNA and RNA analyses was performed to implement modern ‘omic approaches to traditional ecosystem observation programs that examine the diversity, biogeography, and activity of planktonic microbes in the CalCOFI Southern California Bight grid. From 516 DNA molecular marker samples and 184 RNA community transcriptomes microbial diversity and activity were assessed, respectively, during 12 cruises in 2014-2016. Statistical analyses related to the influence temperature, nutrients and mixed layer depth have on microbes indicate taxonomic shifts with respect to the varied environmental conditions, demonstrating a strong linkage between microbial consortia and regional regime. Annotation and richness data of eukaryotic phytoplankton were compared to bulk chlorophyll measurements to provide greater resolution of underlying diversity and found that similar measures of chlorophyll were not indicative of community structure. For example, within the 2015 summer blooms statistically significant (p<0.05) differences in major taxonomic groups, particularly the reduction of centric diatoms was identified, and is instead influenced by pennate diatoms and zooplankton. Molecular data also indicates that community structure within the major phytoplankton groups differs within the distinct nitracline depths (ND) or upwelling regimes with haptophytes and cyanobacteria positively correlated with ND; conversely diatoms, cryptophytes and chlorophytes were negatively associated. Diversity and gene expression spatiotemporal dynamics present a complex grid of microbial and viral communities with variations in the population(s) having more significant impacts on the system, as predicted using empirical dynamic modeling and transcriptional activity profiles. These data add a valuable dimension to current observatories, complementing work on the ecosystem impacts of climate change and ocean acidification.
Abstract Title:
Autotrophic Carbon Fixation Strategies of Nitrifying Prokaryotes in Lakes

Primary Author Block:
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Abstract Body:
Niche specialisation of nitrifying prokaryotes is usually studied by analysing genes coding for key enzymes involved in the oxidation of ammonia and nitrite. The ecological significance of diverse CO2 fixation strategies used by nitrifiers is, however, mostly unexplored. We quantified the distribution of three carbon assimilation pathways used by nitrifiers in eight stratified lakes based on digital droplet PCR and CARD-FISH counts. The spatial distribution of nitrifying organisms using different carbon fixation strategies varied considerably between deep and shallow lakes and showed distinct differences in their vertical distribution within the lakes. Ammonia oxidizing (AO) Thaumarchaeota using the 3-hydroxypropionate/4-hydroxybutyrate pathway were dominating the hypolimnion of deep and oligotrophic lakes, whereas Nitrosomonas related taxa employing the Calvin cycle were the most important AOs in smaller lakes. The occurrence of nitrite oxidizing Nitrospira, assimilating CO2 with the reductive TCA cycle, was strongly correlated with the distribution of AOs. Recently discovered complete ammonia-oxidizing bacteria (comammox) belonging to Nitrospira accounted only for a very small fraction of AOs present at the study sites, although the oligotrophic conditions of the lakes would provide a suitable habitat for this group. Altogether, this study gives not only a first insight on how physicochemical characteristics in lakes are associated to the distribution of nitrifying prokaryotes with different CO2 fixation strategies. Our investigations also demonstrate that functional genes associated with individual CO2 assimilation pathways are suitable markers to study the autotrophic aspect of different guilds of nitrifying microorganisms in environmental samples.
Session Title: AES09 - Freshwater, Wastewater, Drinking Water, and Marine Microbiology: Natural Freshwater and Marine Environments
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Session Primary Track: Applied and Environmental Science
Abstract Control Number: 5440
Poster Board Number: SATURDAY - 846

Abstract Title:
Occurrence and Implication of Acinetobacter baumannii in Water Resources in the Eastern Cape Province, South Africa
Primary Author Block:
M. A. Adewoyin, Black, A. I. Okoh, Black; Univ. of Fort Hare, Alice, South Africa, Alice, South Africa
Abstract Body:
Several Acinetobacter species live in different ecosystems such as soil, freshwater, wastewater and solid wastes, compared to other microbial communities which has attracted intense interests. In this work, we assessed the occurrence of Acinetobacter spp. in three freshwater resources in the Eastern Cape Province, South Africa, including; Great Fish, Keiskemma, and Tyhume rivers, between April and December 2017. A total of 958 presumptive Acinetobacter isolates were recovered from the water samples and screened for the presence of the clinically important Acinetobacter baumannii. Withal, 487 Acinetobacter spp. were identified using polymerase chain reaction techniques and specific primers for Acinetobacter genus. Similarly, a specific primers set targeting the internal 208 bp fragment of the intergenic spacer region of A. baumanni was used for its identification among other species. From the result, 272 (55.6%) isolates were confirmed to be A. baumannii. The presence of A. baumannii in the waterbodies suggests possible contamination of the rivers and also that A. baumannii can thrive well in natural environments. The existence of the pathogen in rivers which are consumed by humans and livestock as well as being used for irrigation system constitute a risk to public health. Keywords: Freshwater, Acinetobacter baumannii, PCR
Giant Flagship Ciliates from Florida, USA: New Records in the Americas for Freshwater and Soil Species Suggest A Global Dispersal

Primary Author Block:
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Abstract Body:
‘Flagship’ ciliates are easily-identifiable, free-living unicellular eukaryotes that play a crucial role in ecosystems. Many of them reach sizes of >1mm. The biodiversity and biogeography of ciliates remains an understudied area of microbial ecology, and ‘flagship’ species represent a way to advance this field. Many flagship ciliates were originally documented in Africa, but had remained unrecorded elsewhere. These ciliates are known from historic texts only, with little ecological information and no photographic record. We hypothesized that these species, claimed to be endemic to Africa, could disperse globally, and that subtropical Florida (USA) was a suitable habitat for such flagship taxa to thrive. We carried out intensive sampling of 100 freshwater habitats using a 500mL bottle sampler to collect samples at various water depths, allowing us to record flagship ciliates thriving in this region. Additionally, soil samples were also collected from 10 sites, incubated (30°C) using an enriched semi-flooded technique to encourage ciliate excystment, and examined daily for 30 days. Our findings thus far include: the first record outside of Africa for several freshwater flagship ciliate species, including the 1.5mm-long Bursaria caudata; and the first records for the Americas of soil species such as Condylostomides coeruleus. This study includes the first data on ecology along with photomicrographs for flagships previously known only from drawings. Our findings demonstrate that ciliates are able to disperse beyond a once hypothesized area of endemism, and likely are able to spread globally. Our discovery of previously-unrecorded flagships from both freshwater ponds and soils in Florida further highlight the potential ease of dispersal for these large single cells. Investigations such as this are important for understanding microbial ecology and biogeography, and provide a dataset for experts and amateurs alike to search for large flagship ciliates within their research areas and to compare distribution at a global level.
Abstract Title:
Diel Trends to Reveal Co-Operative Nutrient Cycling by Freshwater Microbes

Primary Author Block:
A. Linz, K. D. McMahon; Univ. of Wisconsin - Madison, Madison, WI

Abstract Body:
Background: One of the major ecological roles of microbes is the recycling and transformation of nutrients such as carbon, nitrogen, and sulfur. Ecosystem functioning and higher trophic levels depend on the reactions taking place at the microbial scale. In ecology, microbes are often considered a single, stable entity. However, research shows that in freshwater as well as other ecosystems, the microbial community is complex and dynamic, changing on timescales from hours to years. Methods: We hypothesized that sunlight would drive nutrient cycling by initiating a cascade of chemical transformations, with phototrophic microbes becoming active at sunrise. We collected RNA from three lakes with different nutrient regimes (eutrophic, oligotrophic, and humic) every 4 hours to capture these diel trends. We sequenced 109 RNA samples, along with metagenomes from each lake and single amplified genomes to use as references for annotation and classification of metatranscriptomic reads. In total, over two terabytes of data were generated as part of this research. Results: We plan to use gene co-expression from this dataset to identify steps in the carbon cycle that are performed co-operatively. Because algae and cyanobacteria in freshwater release carbon that is then consumed by heterotrophic bacteria, we hypothesized that gene expression in non-phototrophic members of the community would also show display diel trends. We will also use this data to propose cryptic carbon compounds - intermediates in the freshwater carbon cycle that cannot be chemically detected because there is no standing pool in the environment. Conclusions: As metatranscriptomic analysis of freshwater microbes is still rare, this research will provide unprecedented insight into both our hypotheses and a variety of other questions about nutrient cycling in freshwater.
Abstract Title:
Genes Associated with the Fate of Escherichia coli in Water Environment

Primary Author Block:
G. Zheng1, N. Zhang2, M. Reed1, D. Xu2; 1Lincoln Univ., Jefferson City, MO, 2Univ. of Missouri, Columbia, MO

Abstract Body:
Background: Commercial and pathogenic E. coli are known to be able to survive and even grow in water environment outside of their primary habitats. This study was to investigate genetic factors affecting the fate of E. coli in water environment, as it is of great importance to our knowledge of the impact of various E. coli strains on public and environmental health. Methods: Water samples taken from the feces-polluted environment were incubated at 10º C and room temperature (RT) for 4 weeks. E. coli were isolated, using the EPA Method 1603, at day 0 and after 4 weeks of the incubations. Twenty-five isolates from water samples at day 0 and after the 4-week incubations were randomly selected and subject to the whole-genome sequencing. Results: The whole-genome phylogenetic analysis demonstrated that E. coli isolates from the day 0 and from the 4-week incubation at 10º C had a similar distribution of phylogenetic types, while those from the after 4-week incubation at RT were identical, as illustrated by the whole-genome alignment. Furthermore, 37 genes were found to be exclusively associated with the clone of E. coli. Conclusions: The known functions of the genes were determined to be mainly involved with metabolism of polysaccharides, which may play a significant role in the survival of E. coli in water environment.
Abstract Title:
Outstanding Abstract Award: Alterations in Sediment Microbial Communities Due to Hurricane Irma in the Indian River Lagoon, FL, USA
Primary Author Block:
D. J. Bradshaw, II, P. J. McCarthy; Florida Atlantic Univ. - Harbor Branch Oceanographic Inst., Fort Pierce, FL
Abstract Body:
Natural disturbances such as hurricanes can have a massive impact on environmental parameters in coastal waters. Hurricane floodwaters have an immediate impact by causing low salinity, high turbidity, and enrichment of nutrients and organic matter, while the sustained drainage of rainfall from inland can lead to low salinity for an extended period of time and the introduction of contaminants. The effects of hurricanes on the taxonomy of sediment microbes remains largely unexplored. The data presented here is from three surveys in 2017 (August 31st, September 15th, and October 31st) at four sites in the Indian River Lagoon, FL. Hurricane Irma hit Florida on September 10th as a Category 3 storm. In Fort Pierce, FL the storm produced 15 to 21 inches of locally heavy rainfall and a record daily rainfall of 13.08 inches. This caused precipitous drops in water salinity (>15ppt) and porewater salinity (>13ppt) at all sites. One site had marked changes in sediment parameters increasing Total Organic Matter (1.80% - 21.03%), water content (27.27% - 83.57%) and Enterococci counts (0 - 1,569 cfu/mL). Enterococci at the other sites increased to a lesser extent (119-287 cfu/mL). We used Illumina MiSeq next generation sequencing to determine the composition of the microbial communities present at these sites and analyzed the sequences with QIME, R, and PRIMER7. The microbiome showed major shifts in composition between the three sampling events corresponding to changes in sediment characteristics and salinities. This project provided a unique opportunity to study how the immediate effects of hurricane-related rains and runoff can change the composition and ecosystem functions of microbial communities. Alterations in the levels of microbial community members are related to environmental parameters allowing them to act as sensitive indicators of environmental health. This study allowed us to see the effects of sustained water drainage on prokaryotic communities that can be used by agencies to inform decisions regarding water management.
Abstract Title:
High-Resolution Spatial-Temporal Dynamics of Escherichia coli in An Urban Stream System

Primary Author Block:

Abstract Body:
In urban landscapes, aging sewage infrastructure, leaky sewer lines, failing septic systems, and increasing amounts of impervious surfaces can impair water quality and affect public health via introduction of enteric pathogens into surface waters. Detecting these impacts requires active surveillance. In this study, a network of stream sites around Athens, Georgia (USA), an urban area home to ~100,000 people served by sewers (>100 years old), septic systems, and bounded by agricultural land, were sampled weekly for Escherichia coli, a fecal indicator bacteria, over a 15-month period (October 2016 to December 2017). Over this sampling campaign, 228 stream samples were collected at 9 stations. For all samples collected, E. coli concentrations were determined using EPA Method 1603. Over the 15-mo period, E. coli counts averaged 1,055 CFU 100 ml-1 and ranged from a low site average of 444 CFU 100 ml-1 (n = 25) in residential areas to a high site average of 1,747 CFU 100 ml-1 (n = 28) on the University of Georgia campus. Additionally, two sites immediately downstream of a hospital sewer line had average E. coli levels of >1,300 CFU 100 ml-1 (n = 28,39) as well as the highest single sample value of >12,000 CFU 100 ml-1; although numbers were high year-round, the highest counts were observed during spring and early summer suggesting consistent inputs of fecal material. Additionally, a synoptic sampling campaign was conducted across 40 stations to assess site variability within a 4 h time window (November 2017). In this collection, E. coli averaged 2,046 CFU 100ml-1 (ranging from 270 to 9,550 CFU 100 ml-1). Synoptic samples were also analyzed for extended spectrum beta lactamase (ESBL) producing enterobacteriaceae using chromogenic agar followed by PCR. E. coli isolates PCR-positive for both blaCTX-M (cefotaximase) and blaTEM (penicillinase) genes were detected in an urban stream segment (n=2) and in wastewater influent (n=1). A blaCMY (carbapenemase) positive E. coli isolate was also detected in an adjacent stream site. These preliminary data point to possible antibiotic resistance activity in urban waters.
Abstract Title:
Iterative Subtractive Binning of Freshwater Chronoseries Metagenomes Recovers Nearly Complete Genomes from over Four Hundred Novel Species

Primary Author Block:
L. M. Rodriguez-R, D. Tsementzi, C. Luo, K. T. Konstantinidis; Georgia Inst. of Technology, Atlanta, GA

Abstract Body:
The detailed study of natural microbial communities in most environments has been traditionally hindered by the limited number of genome sequences that can be recovered. The scarcity of reference material to identify microbial populations is therefore a major obstacle in current microbial ecology research, with a few exceptions such as communities with extremely low-diversity and cases where large collections of reference genomes exist. Recently, this issue has attracted special attention due to the availability of methods for the reconstruction of genomes from metagenomes, or binning. A small number of large-scale efforts to recover genomes from previously uncharacterized microbial species have recently yielded collections representing novel deep-branching clades in the tree of life. Here, we leverage a chronoseries consisting of 69 metagenomes from seven sites along the Chattahoochee River (Southeastern USA) including five lakes and two estuarine locations. We developed an iterative binning methodology gradually decreasing sample diversity to maximize the number and quality of recovered genomes. Our workflow consists of de novo sample clustering, co-assembly and sub-assembly by sample group, binning, and genome quality evaluation. Once a group of high-quality genomes is identified, we filter out reads derived from that set through mapping, and iterate the procedure until we observe no significant gains in genome sequences or phylogenetic diversity. In the first iteration we recovered 199 high-quality genomes from 176 de-replicated groups (clades with Average Nucleotide Identity-ANI ≥ 95%). After eight iterations, this number increased to 1,126 genomes from 463 groups, with a concomitant increase in community fraction from ~15% to 40-50%. Moreover, this collection represents a set of largely uncharacterized groups at high taxonomic ranks. Using the Microbial Genome Atlas (MiGA), we were able to confidently classify only eight genomes at species or genus levels using the NCBI Genomes database as a reference. 216 genomes were classified at order level or below, and the majority of genomes in the set (545) were classified only to class level. An additional 295 genomes potentially represent members of novel classes classified at phylum level, and 70 genomes potentially represent novel phyla. Finally, we were able to identify cases of seasonal rhythmicity and different levels of endemism in our collection, demonstrating quantitatively similar effects of seasonality and biogeography on freshwater microbial community assembly.
Abstract Title:
Bacterial and Viral Community Dynamics During Bloom Seasons in Lake Eire and An Inland Lake

Primary Author Block:
S. Lee, J. Lee, I. Mrdjen; The Ohio State Univ., Columbus, OH

Abstract Body:
Background: Anthropogenic nutrient loading and environmental changes increase the frequency and severity of cyanobacterial bloom events. Excessive growth of cyanobacteria and cyanotoxins have negative impacts on environmental and public health. However, the ecology of bloom dynamics, especially cyanobacteria and cyanophages, in lakes is poorly understood. The objective of this study is to understand the ecological dynamics of cyanobacteria and cyanophage communities, together with microcystin variations, during bloom seasons in western Lake Erie and Buckeye Lake, Ohio, USA.

Methods: Water samples were collected from both lakes between June and September 2016, and analyzed for water quality parameters, including nutrients, phycocyanin, chlorophyll-a, and microcystins. A metagenomic analysis was performed to determine bacterial and viral communities and their diversity. Total cyanobacteria and toxin producing cyanobacteria were also quantified with quantitative PCR. Results: The concentrations of chlorophyll-a and microcystins were higher than the WHO and USEPA eutrophication guidelines for recreational activities and drinking water. The results of bacterial community analysis at the phyla level show that Proteobacteria and Cyanobacteria were dominant in both lakes. However, the dominant genus within the cyanobacteria group was different: Microcystis dominated in Lake Erie and Planktothrix in Buckeye Lake. The qPCR analyses also confirmed that the level of the Microcystis toxin gene was higher than the Planktothrix toxin gene in Lake Erie while Buckeye Lake had more Planktothrix toxin genes than Microcystins toxin genes. In both lakes, the virus community was dominated by Podoviridae that have a large bacterial host range. Conclusions: This is the first study characterizing a cyanophage community in both lakes. These findings can enhance our understanding of their biology and characteristic complexes, and clarifies microbial interactions and structure in bloom-affected western Lake Erie and other inland lakes.
Rivers support growth of pathogens such as Salmonella, the leading cause of bacterial food-borne illness in the United States. While often connected with agricultural land use practices such as poultry and cattle farming, Salmonella is also associated with wildlife and sewage, which can impact microbial river ecology. Salmonella enterica is a very diverse bacterium, separated into >2,500 serovars. Not all serovars are created equal; some are more frequently associated with human illness than others, and they can also differ in their resistance to antibiotics. CRISPR-SeroSeq is a powerful new molecular technique for high-resolution characterization of Salmonella serovar composition in a single sample. To investigate the Salmonella serovar diversity in rivers, we chose 60 sites within a small watershed in Adams County, PA that includes three creeks: Marsh Creek, Willoughby Run, and Rock Creek. These converge to become the Monocacy River, which drains into the Potomac River and empties into the Chesapeake Bay. Collection sites were chosen so that they were directly upstream and downstream of potential Salmonella reservoirs (pastures, sewage facilities, etc.). To assess any seasonal influence, two collections were made, November 2016 and April 2017. Salmonella was identified at ~90% of sites, with a slightly more positive samples collected in the fall than in the spring. CRISPR-SeroSeq revealed multiple serovars in >60% of samples, and up to six serovars in a single sample. Salmonella serovars Enteritidis and Kentucky were most prevalent, found in 77% and 66% of samples, respectively. Serovars Heidelberg and Typhimurium were both found in 20% of samples and mostly in the fall. Other serovars identified were Newport (0.5%) and Hadar (0.8%). Serovars Schwarzengrund, Cerro and Thompson were each found at single sites. Of nine different serovars detected, five are in the top 20 that cause human illness. Importantly, with the exception of serovar Enteritidis, pathogenic serovars were not always the most predominant in our samples, suggesting they may not have been detected had a lower resolution approach been used. Serovars Kentucky and Cerro, while typically not responsible for salmonellosis, are often found in poultry and cattle, respectively, and multiple multidrug resistant isolates of these serovars have been found in the United States. This work highlights the potential public health threat that rivers pose as ecological reservoirs for Salmonella and reveals a high level of serovar diversity in single water samples.
Abstract Title:
Metagenomic Analysis of Microbiome Communities in Different Marine Sediments Along Tolo Harbour, Hong Kong

Primary Author Block:
J. Chen1, S. McIlroy1, A. Anand1, D. Baker1, G. Panagiotou2; 1the Univ. of Hong Kong, Hong Kong, Hong Kong, 2Hans Knoell Inst., Jena, Germany

Abstract Body:
Microorganisms living in the marine sediment can be regarded as important bioindicators in relation to the biogeochemical process of the benthic ecosystem because they are playing important roles in important organic cyclings such as carbon, sulfur and nitrogen. Therefore, scientists are focusing on revealing the relationships between microbiome communities and environmental conditions inside the sediments with either molecular techniques or high-throughput sequencing. We have performed a comprehensive metagenomic analysis on the microbial communities inside the marine sediments of four sites along Tolo Channel in Hong Kong for uncovering both the taxonomic and functional profiles of micro-communities under different pollutant conditions due to human activity. Twelve marine sediment samples were collected in four sites (Centre Island, Che Lei Pai, Port Island and Tung Ping Chau respectively) along Tolo Channel and were subjected to metagenomic shotgun sequencing. After passing through the standard quality control, the filtered sequencing data were used for both prokaryotic and eukaryotic taxonomic profiling at different levels. Further taxonomic analysis including identifying the potential pathogenic genus and species, calculating the microbial diversity and estimating the replication rate of top abundant species were performed. Besides, the functional annotation of antibiotic resistance genes (ARG), biosynthetic gene clusters (BGC) and KEGG orthology (KO) were implemented. The analysis was performed using our in-house computational pipelines. Our study provide evidence that the microbial communities along with the major organic cycling functions would be influenced by different pollution level and the human activities can accelerate the dissemination of antibiotic resistance. Furthermore, our study can provide the government with valuable suggestions for marine environmental protection.
Abstract Title:
Multiple Factors Drive Patterns of Ammonia-Oxidizing Communities in Louisiana Salt Marshes

Primary Author Block:
A. Bernhard1, A. Giblin2, B. Roberts3; 1Connecticut Coll., New London, CT, 2Marine Biological Lab., Woods Hole, MA, 3Louisiana Universities Marine Consortium, Chauvin, LA

Abstract Body:
Background: Microbial community dynamics can be influenced by many factors, including both natural and human-induced. Methods: To identify factors driving nitrifying microbes in salt marshes following the Deepwater Horizon oil spill, we characterized communities of ammonia-oxidizing archaea (AOA) and bacteria (AOB) from salt marsh sediments in the Gulf of Mexico. Samples were collected from oiled and unoiled sites in July from 2012-2016 from east and west Barataria Bay (EB and WB), and Terrebonne Bay (TB) on the Louisiana coast. AOA and AOB abundance was determined by quantitative PCR of the archaeal and betaproteobacterial amoA genes and community composition was assessed using TRFLP and DNA sequencing of amoA genes. Results: No consistent oil effect was detected for abundance or community composition for either AOA or AOB. AOA abundance showed significant interannual variation in TB and WB, but abundance was stable and significantly higher in EB. AOB abundance was more variable compared to AOA, and was significantly higher at WB and TB relative to EB. AOA communities were significantly different for all three regions, but all were dominated by amoA genes related to Nitrosopumilus maritimus. AOB communities were also significantly different in all regions, with Nitrosomonas-related amoA genes dominating in TB and EB, and Nitrosospira-like amoA genes dominating in WB. Interestingly, AOB abundance and both AOA and AOB diversity decreased in 2014 in all three regions. Conclusions: Local heterogeneity in sediment chemistry (e.g. organic C, total N, and salinity varied by region) is likely the main driver of community composition and abundance for AOA and AOB in these marshes, although there is evidence, in some cases, of broad-scale drivers that impact abundance and diversity across all regions synchronously. Results from this study provide important insights about how large-scale disturbances impact nitrifying microbes in the Gulf of Mexico, and suggest that nitrogen cycling may be controlled by local factors within regions, but during some periods, large-scale drivers might override these localized differences.
Abstract Title: Prokaryotic Species Diversity in Great Smoky Mountains Natl. Park: from 16s Rdna to Whole Genomes

Primary Author Block:
S. P. O’Connell, T. K. Carlson, L. M. Dye, K. R. Fraser, R. P. McKinnon; Western Carolina Univ., Cullowhee, NC

Abstract Body:
We have been sampling the microbial diversity of Great Smoky Mountains National Park (GSMNP) for over 15 years. Much of this work attempts to understand bacterial and archaeal distributions in unique habitats such as the rhizosphere of Eastern Hemlock (Tsuga canadensis), elk rumens (Cervus elaphus), and in stream waters and soils. Recently, the genomes of four bacteria from a stream were sequenced in order to verify their uniqueness. Over 500 cultured and 1,200 clones have been documented in our work and compared using sequences of 16S rDNA. Classifications for the species have been made using Classifier and SeqMatch tools within the Ribosomal Database Project (RDP) and 15 total phyla of bacteria and archaea have been encountered. The cultures have been dominated by Proteobacteria, Firmicutes, and Bacteroidetes with the genera Paenibacillus, Streptomyces, and Pseudomonas particularly common. The clones show a very high and diverse distribution of Acidobacteria in soils and rhizospheres as well as many genera from the Proteobacteria. Ammonia oxidizing archaea were also common. Using RDP results and phenotypic testing, four cultures including three from the Enterobacteriaceae and one from the genus Paenibacillus, were shown to be low matches to known species. These cultures had their whole genomes sequenced using an Illumina HiSeq 2500 system. Gene annotations and alignments to other sequenced genomes showed that one matched a known species (Erwinia billingiae), two were probably new genera within Enterobacteriaceae, and one was a novel species of Paenibacillus. Our work in GSMNP corroborates high biodiversity shown in GSMNP for other taxa, e.g., in salamanders, plants, and insects. It also appears that soils may be dominated by Acidobacteria, which is unusual compared to other soils around the globe. Whole genome sequencing supports our hypothesis that 16S rDNA can predict the novelty of bacteria and it gives additional tools to examine (e.g., genes for pathogenicity, phages, resistance to metals and antibiotics, transposons; and for employing unique biochemical reactions) that may give us broader insight into the biology of these organisms in situ. Such information will be of great value to Park managers and to microbiologists interested in discovering links between genes and ecology in a natural setting.
Abstract Title: Full Year Assessment of Recreational Waters in Papago Park Using Microbial Source Tracking

Primary Author Block: R. Botello, B. Charlton; Grand Canyon Univ., Phoenix, AZ

Abstract Body:
Recreational waters such as ponds, rivers and lakes are expected to be in accordance to local and national standards in regards to water quality. There are measurable pathogen indicators such as E. coli that can show whether a body of water is safe to the public or not. It is especially important to identify sources of contamination if one is present. Microbial source tracking methods using Quantitative Polymerase Chain Reaction (qPCR) is crucial in identifying sources of fecal contamination so control measures can be implemented to protect public accessible waters. This study aims to identify sources of fecal contamination and determine seasonal trends of microbial indicator bacteria in pond 1 and canal. Papago Park located in Phoenix, Arizona, is home to popular hiking destinations and tourist attractions just east of the Phoenix Zoo. The canal is serviced by the Salt River Project (SRP), Phoenix’s largest utility company that provides power and water for over a million residents. The canal drains into the park supplying water to all three ponds within the park. These ponds are accessible to the public and are often used for fishing and other recreational activities year-round. Samples were tested for total coliform and E. coli by using the Colilert Quanti-Tray 2000 method. Preliminary data shows E. coli concentration threshold at a peak of 61.3 MPN/100mL for the canal and 365.4 MPN/100mL for pond 1. Despite detecting E. coli concentrations, this method does not identify the actual sources of contamination. Therefore, the samples were further analyzed using Microbial Source Tracking. Preliminary data indicates both canal and pond 1 are positive for Human Bacteroides (Human) with 75% (n=26) and 100% (n=26) Total Bacteroides (Total). Determining water quality is necessary in establishing whether or not the public may be at risk of serious gastrointestinal diseases. Some of which include Cryptosporidium and Giardia, exposure may result in severe diarrhea, fever, vomiting, and nausea among other symptoms. The results from this study will be presented to city officials if results indicate the public is at risk. Remediation steps may include proper signage, informational pamphlets about fishing techniques and disposal of waste.
Abstract Title:
Microcosm Studies of Enterococci and Chlorophyll in the St. Lucie, Loxahatchee, and Lake Okeechobee Watersheds

Primary Author Block:
E. Kelly1, M. Gidley2, C. Sinigalliano3, L. Brand4, H. Solo-Gabriele1; 1Univ. of Miami, Coral Gables, FL, 2Univ. of Miami CIMAS, Miami, FL, 3NOAA, Miami, FL, 4Univ. of Miami, Miami, FL

Abstract Body:
In south Florida, controversy has surrounded the cause of coastal algal blooms; Lake Okeechobee discharges are cited by many environmental groups, while agricultural interests implicate septic tanks. Earlier work by our team (Donahue et al. 2017) indicated that Florida beaches with rivers inside 600 m of sampling sites had statistically higher fecal indicator bacteria (FIB) exceedances compared to those without riverine influence. Two of the highest exceedances in the state were at Dubois Park (Loxahatchee River, not connected to Lake Okeechobee) and Roosevelt Bridge (St. Lucie River, connected to Lake Okeechobee). Both are monitored for FIB as part of the Florida Healthy Beaches program. Releases from the lake are sent downstream during rainy periods to control flooding in the region near the lake; in fall 2015, Florida experienced an unusual wet period during the normal “dry season.” FIB spikes were reported at both Dubois and Roosevelt Bridge, and blue-green algae was observed downstream near Roosevelt Bridge. Microcosm pilot studies that analyze Lake Okeechobee water and substrate from our lake sampling site were initiated in September 2017 to evaluate the impacts from Lake Okeechobee on enterococci (FIB) and chlorophyll (microalgae). These studies are done as part of a year-long study evaluating the FIB and nutrients at the three sites. Our hypothesis was that water and/or sediment from Lake Okeechobee may be contributing to elevated FIB and algae. Dubois Park was used as a control. To isolate the impacts from Lake Okeechobee, we analyzed 1) water only from Lake Okeechobee and Dubois Park, 2) Lake Okeechobee water and substrate, and 3) Lake Okeechobee water with either Lake Okeechobee substrate or Dubois Park sand. Light and temperature were optimized for either FIB or algal growth. In the water-only microcosms, enterococci did not survive past 12 hours from either site. With Lake Okeechobee substrate, chlorophyll and pheophytin were higher and enterococci persisted through 36 hours. FIB levels in the third study were higher at the beginning, dropped by 12 hours, and spiked at 24 hours. This work indicates that our next experiments should investigate the role of sediments and include our nutrient data from the main study, to understand if they encourage the persistence and potential regrowth of FIB. Future studies should focus on the characterization and evaluation of the native sediment, organic matter, and the sediment transport in all three watersheds, to understand whether Lake Okeechobee sediments may be contributing to algal blooms downstream.
Session Title: AES09 - Freshwater, Wastewater, Drinking Water, and Marine Microbiology: Natural Freshwater and Marine Environments
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 7347
Poster Board Number: SATURDAY - 860

Abstract Title: Assessment of Proposed Marine Mammal Water Quality Indicator Organisms in A Recirculating Artificial Seawater System

Primary Author Block:
C. N. Owen1, J. B. Rose1, W. Van Bonn2, S. Manning1, C. Edwardson2; 1Michigan State Univ., East Lansing, MI, 2Shedd Aquarium, Chicago, IL

Abstract Body:
Institutions that house marine mammals are required by law to monitor total coliform counts in enclosure waters and maintain counts ≤ 1,000 colony-forming units (CFU) per 100 mL. These regulations are a driver of management practices such as water oxidation. A proposal to reduce the limit to 500 CFU/100 mL and add limits for Enterococcus, Pseudomonas, and/or Staphylococcus underscores the need to evaluate their use as indicators of water quality for marine mammals. The goals of this study were to evaluate baseline colony counts and physical distribution of these organisms over a five-day period in a recirculating artificial seawater system that houses cetaceans and pinnipeds and utilizes ozone disinfection. Colony counts were performed on water from the exhibit as well as system plumbing immediately upstream and downstream of ozone contact using m Endo LES, mEI, m PA-C and Baird-Parker (BP) media to test for total coliforms, Enterococcus, Pseudomonas, and Staphylococcus, respectively (15 counts per medium at each site). Feces and chuff from 5 dolphins in the exhibit were also tested for the presence or absence of growth on these media. 111 colonies were isolated and classified using 16S rRNA gene sequencing. 60% of dolphin feces and 80% of dolphin chuff showed growth on mEI, m PA-C and BP, while 100% of feces and 60% of chuff showed growth on m Endo LES. Among sequenced colonies, 17 of 41 colonies (41%) from m Endo LES were non-Enterobacteriaceae, 0 of 27 (0%) from mEI were non-Enterococcus, 13 of 16 (81%) from BP were non-Staphylococcus, and 24 of 27 (89%) from m PA-C were non-Pseudomonas. Except for mEI, these results suggest that alternative methods should be adopted for accurate testing of similar systems in the future. Colony counts on all media types were lowest downstream of ozone contact, while differences between colony counts in the exhibit vs. upstream of ozone contact varied between tests (see graphic).
Abstract Title:
Chemical and Microbial Analysis of Recreational Freshwater in and Around Blue Marsh Reservoir, Berks County, Pa

Primary Author Block:
Z. T. Weagly, J. M. Felker, T. H. Mysliwiec; The Pennsylvania State Univ., Reading, PA

Abstract Body:
Human use areas along selected waterways expose individuals to bacterial populations with unique biochemical characteristics and potential pathogenic properties. Initial visual observations from three sites (upstream, downstream, and reservoir) along a heavy human use waterway in central Berks County, Pennsylvania indicated that different regions of the same creek comprise different levels of algal growth, invertebrates, and plant life. The working hypothesis for this study stated that the downstream site would have a vastly different microbial population from the upstream site. Testing was performed on these areas to determine differences in the water chemistry and the microbial populations. Chemical testing included; pH, temperature, NO3-, PO43-, and BOD. Dramatic variations in pH were observed and key differences in nitrate levels were found for all sites tested. Microbial tests included determination of preferential food sources, relative abundance, and overall colony forming units (CFU’s). CFU’s were determined using three independent plate counts looking for Escherichia coli, heterotrophs, and Enterococcus spp. Results from the CFU’s testing at each location varied seasonally, both amongst the different and within each site. Initially the upstream location contained higher counts of CFU’s for all species tested, however, the collection site within the reservoir, where human use was highest, showed higher counts after the warm season. As expected, human use areas were shown to have higher counts of E. coli and Enterococcus spp. BioLog Ecoplates were inoculated to examine variation in biochemical utilization by the microbial populations at all sites and to determine the preferred carbon sources of local microbial populations. Ecoplate results identified the presence and apparent preference for synthetic carbon sources as primary metabolites. Analysis identified Tween 40 and Tween 80 as two preferred carbon sources. Both have been identified as chemicals which can limit the effectiveness of certain antibiotics used to combat bacterial infections. The presence of this particular carbon source is likely due to common agricultural practices in the area. 16S ribosomal sequencing of select samples is currently underway.
Abstract Title:
Characterization of A Novel Purple Non-Sulfur Bacterium for Bioremediation of Petrochemical Wastewater

Primary Author Block:
A. J. Walters, D. C. Porter, C. C. Riddle, R. C. Sims, C. D. Miller; Utah State Univ., Logan, UT

Abstract Body:
The petroleum industry extracts 378 billion gallons of crude oil and produces 4.3 billion gallons of produced water (PW) and petroleum refinery wastewater (PRW) each day. Upflow Anaerobic Sludge Blanket (UASB) reactors hold potential for the bioremediation of these wastewaters by transforming the waste into valuable resources including methane and hydrogen gas. This study investigated the potential for PRW bioremediation by purple non-sulfur bacteria isolated from a UASB reactor. The bacterium was isolated on 2% succinate media and identified using 16S rRNA sequencing with primers 338F and 1390R. A BLAST analysis found the 16S sequence to be 100% identical to Rhodopseudomonas palustris, which can transform polluted wastewater into hydrogen gas, bioplastics, and bioelectricity. In a pilot study, 8 triplicates of undiluted PRW were prepared in serum vials anaerobically, 24 samples total. All samples were autoclaved, then some were filtered to remove sediments and spiked with benzene compounds (BTEX). Therefore, two triplicates of each of the four sample types were prepared: 1) Unfiltered, without BTEX. 2) Unfiltered, with BTEX. 3) Filtered, without BTEX. 4) Filtered, with BTEX. One set of four triplicates was allowed to self-digest as a negative control. 1 mL pure culture of the isolate was added anaerobically to the others. Reduction in chemical oxygen demand (COD) was monitored to assess treatment effectiveness over time using HACH High Range kits. The bacteria were able to significantly reduce the COD as compared to negative controls. After a 2 tailed homoscedastic T-Test, there was no significant reduction of COD in the controls, while the samples containing the isolate significantly reduced the COD (p-value <0.05). On average, the COD was reduced by 1.4% in the negative controls and by 35.4% in the inoculated subjects (p-value <0.001). One triplicate of filtered PRW without BTEX reduced the COD from 335 ± 33 to 188 ± 1 mg/L, a 44% reduction (p-value <0.05). The corresponding control reduced the COD from 211 ± 30 to 201 ± 6 mg/L, a 5% reduction (p-value = 0.66). These results shown indicate that this novel isolate merits further investigation. As the environmental isolate was able to grow on raw PRW and significantly reduce the COD as compared to a negative control, more stringent characterization of treatment effectiveness on produced water and petroleum refinery wastewater is warranted. As this organism becomes better understood it can be optimized for use in bioremediation of wastewater and production of bioproducts.
Abstract Title:
Towards Developing A Genetic Sys. for the Brown Tide Bloom Forming Pelagophyte Aureococcus Anophagefferens

Primary Author Block:
E. R. Gann, B. C. Calfee, T. Chen, E. R. Zinser, T. E. Sparer, T. B. Reynolds, S. W. Wilhelm; The Univ. of Tennessee, Knoxville, Knoxville, TN

Abstract Body:
The eukaryotic brown alga, Aureococcus anophagefferens, has bloomed annually in the Northeast United States since the 1980s. Although the blooms are not toxic to humans, brown tide blooms cost the United States millions of dollars due to toxicity to bivalves, and destruction of fisheries and seagrass due to severe light attenuation. These brown tide blooms have since spread globally, which has been hypothesized to be caused by transport in ballast waters of ships. Aureococcus possesses a 56 Mbp genome, containing a large suite of genes, from diverse sources, hypothesized to aid in bloom proliferation in shallow bays. The importance and function of many of these individual genes has not been elucidated due to the inability to genetically modify Aureococcus. To be able to address these environmentally relevant questions, we have been developing tools to make this a genetically tractable system. Initial efforts have used optimized electroporation conditions to introduce DNA into the cells. To select for potential transformants we have developed constructs to express green fluorescent protein, or a Nourseothricin resistance marker. Transformants have been detected by both an increase in fluorescence by flow cytometry, and the ability to survive lethal concentrations of nourseothricin. Both linearized and circular DNA electroporated into Aureococcus has led to a expression with transformation efficiencies of ~1 in 108 cells transformed, which was lost after a single culture transfer. Our results provide an insight into the necessary steps to develop Aureococcus as a new genetic system and tool for marine microbial research.
Quality Assessment of Herbal Medicines and Products with Reference to Selected Microbial Isolates

Primary Author Block:
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Abstract Body:
Herbal medicines and herbal products have been used extensively across the world, which has created important public health issues. In this scenario, this study has concentrated on the microbial quality of different herbal medicines and herbal products. This descriptive and inferential study was carried out to determine the microbial quality of herbal medicines and herbal products available in the market of Kathmandu valley, Nepal. The test procedure was based on the "WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues" with few modifications. The results showed that among 114 herbal samples processed, 50.8% samples were contaminated with bacteria whereas 40.3% samples were contaminated with fungi. Furthermore, 33.3% of the samples had the presence of pathogenic microorganisms. All locally available samples were contaminated whereas 12% of the branded samples were contaminated with pathogenic microorganisms. The pathogenic microorganisms found were Staphylococcus aureus, Pseudomonas aeruginosa, Clostridium perfringens and Escherichia coli. The isolated pathogenic bacteria showed the different pattern of resistance to various types of antibiotics. Here, 39.5% of the samples did not comply with the limits as stated by WHO guidelines. A positive association was found between the presence of pathogenic microorganisms with the type of sample, WHO intended use of herbal material and branded or local type (p<0.05). There is a need for regular monitoring and quality check of herbal medicines and herbal products available in the market of Kathmandu valley. Consumer and producer should be aware whereas the concerned authorities should be responsible for the microbial quality of herbal medicines/products.
Abstract Title:
The Inhibitory Effects of Essential Oil Constituents against Germination, Outgrowth and Vegetative Growth of Spores of Clostridium Perfringens Type A in Lab. Medium and Chicken Meat

Primary Author Block:
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Abstract Body:
C. perfringens type A is the causative agent of C. perfringens type A food poisoning (FP) and non-foodborne (NFB) human gastrointestinal diseases. Due to its ability to form highly heat-resistant spores, it is of great interest to develop strategies alternative to thermal processing to inactivate C. perfringens. Thus, in this study we evaluated the inhibitory effects of essential oil constituents (EOCs) (cinnamaldehyde, eugenol, allyl isothiocyanate (AITC), and carvacrol) against germination, outgrowth and vegetative growth of spores of C. perfringens FP and NFB disease isolates in laboratory medium and chicken meat. The cinnamaldehyde, eugenol and carvacrol, but not AITC, all at 0.05-0.1%, inhibited the germination of spores of all tested C. perfringens isolates in Tripticase-glucose-yeast extract (TGY) medium. Furthermore, all tested EOCs at 0.05-0.1% arrested the outgrowth and vegetative growth of C. perfringens spores in TGY, with AITC and carvacrol being the most effective. However, among four tested EOCs, only AITC (at 0.5%-2.0%) was able to inhibit the growth of C. perfringens spores in chicken meat and no such inhibitory effect was observed even with a 10-fold higher concentration (5%) of carvacrol. In conclusion, our current work identified AITC as an effective EOC to control spores and vegetative cells of C. perfringens isolates in laboratory medium and chicken meat. Further studies on evaluating the effectiveness of different combination of EOCs against C. perfringens spore growth in different meat products should establish an effective use of EOCs to control the risk of C. perfringens-mediated illnesses.
Abstract Title:
The Effect of Ginger Extract, Wild Blueberry Extract, and Polysorbates (Ps20, Ps80) on Pseudomonas Aeruginosa Biofilm Formation In-Vitro

Primary Author Block:
M. Miari, S. S. Rasheed, N. Haidar-Ahmad, A. Abou Fayad, G. M. Matar; American Univ. of Beirut, Beirut, Lebanon

Abstract Body:
Background: Biofilms are a group of bacterial cells that are embedded in a matrix of extracellular polymeric substances (EPSs) and communicate via a quorum sensing system (QS). Pseudomonas aeruginosa is a strong biofilm forming pathogen that in fact poses a challenge on a variety of clinical and industrial settings. Therefore, many natural products and surfactants were screened and valued for their antibiofilm capacity. In this study we assessed the inhibitory effect and mechanism of action at the molecular level of ginger extract (Zingiber officinale Rosc.), wild blueberry extract (Vaccinium angustifolium), and polysorbates (PS20/PS80) on biofilm formation. Methods: Ginger and wild blueberry extraction was done using ethanol and distilled water, respectively. Hexane and methanol were then used for extracts’ liquid-liquid portioning. LC-HRMS was also carried out to obtain extracts’ fractions. The effect of the crude extracts, fractions, and polysorbates on the growth and biofilm of Pseudomonas aeruginosa isolate (PAN14) was assessed. Transcription levels of biofilm encoding genes ndvB, pelC, algC and quorum sensing genes lasI, lasR, rhlI, and rhlR, were evaluated by RT qPCR in treated wells where biofilm inhibition was observed. Results: Growth curves revealed that extracts and polysorbates’ concentrations used did not impact P. aeruginosa growth. The 96 well microtiter plate biofilm assay showed a reduction in biofilm when 5% ginger, 25% wild blueberry extracts, 0.2% of polysorbate 20, and 0.25% of polysorbate 80 were added. LC-HRMS analysis of ginger extract has shown an abundance of gingerol in the hexane layer. However, wild blueberries’ chromatograms have shown an abundance of various constituents that differ between wild blueberry’s peel and pulp and wild blueberry’s pulp extracts. Rt-qPCR results showed a decrease in transcription levels of exopolysaccharide and quorum sensing genes in biofilm treated wells with a highest reduction of 363.6 folds in ndvB gene upon treating with 25% wild blueberry’s peel and pulp extract. Conclusions: The decrease in the relative gene expression of biofilm forming and quorum sensing encoding genes shed the light on the mechanism of action of ginger and wild blueberry’s constituents as well as polysorbates 20 and 80 on P. aeruginosa biofilm formation. Future experiments using mouse models are useful to test this biofilm inhibiting ability in-vivo.
Synergistic Activity Potential of Stevia rebaudiana WholeLeaf Extract against Environmental Bacteria

Primary Author Block:
G. Khadka, J. Dominguez, K. Jayachandran, K. Shetty; Florida Intl. Univ., Miami, FL

Abstract Body:
There is an increased interest in antimicrobial potential of Stevia rebaudiana (natural sweetener) leaf extracts and its application in pharmaceuticals and preservatives. In addition, it is also being observed that stevia extract can also potentially act synergistically in combination with antibiotics. Bacteria are unlikely to develop resistance against plant extracts that are mixture of active compounds. Potential synergistic effects of stevia in combination with antibiotics may help reduce antibiotic dose. The potential effects on environmental organisms from antimicrobial and synergistic effect of Stevia are not known. In this study, we evaluated the effectiveness of whole leaf stevia extract in combination with Streptomycin against Rhizobium leguminosarum (NRRL B-509) and Escherichia coli (NRRL B-2207) in vitro. The susceptibility of the bacteria was evaluated by growth assay (optical density) using multi-well plates for the period of 24 hours. The bacteria were grown in dilute nutrient broth. The whole stevia extract in water included four concentrations (20 mg/ml - 20 µg/ml) of Splenda Naturals ® (A) and Stevia in the raw ® (B) in combination with 10 µg/ml of streptomycin. The results appeared to indicate that the presence of sugar or sugar alcohol components in the extract interfered with the inhibitory effect of stevia extract at higher concentration. Synergistic inhibitory effect on both Rhizobium and E. coli growth was observed only with Stevia product B at concentrations below 2 mg/ml. There was a 35% growth inhibition compared to the streptomycin only treatment in both R. leguminosarum and E. coli exposed to stevia concentrations 20 mg/ml and 20 µg/ml respectively. Results from this study show that Stevia leaf extract has potential for synergistic antibacterial activity on environmental bacteria. Additional studies using pure stevia extracts without added components and in combination with different antibiotics needs to be explored.
Abstract Title:
Anti-Microbial Potentials of Of N-Hexane Extracts of Alligator Pepper, A Party Snack in Africa

Primary Author Block:
J. I. Odimegwu, B. Asabisi, M. Sodeinde, M. O. Ilomuanya; Univ. of Lagos, Akoka, Nigeria

Abstract Body:
Alligator pepper (Ap) so called because of the gator-like shape of the fruit (pod) is scientifically named Aframomum melegueta. The seeds are used by local communities of Africa to treat and manage microbial infections. It is a popular party snack at weddings and local social meetings of people where possibilities of food poisoning are rampant. We evaluated antimicrobial potentials of Ap seeds on selected microbials; Staphylococcus aureus, Salmonella, S. typhi, Escherichia coli, and S. dysenteriae that cause food poisoning using Metronidazole and Gentamicin as standards. The effectiveness of Metronidazole as antidote to food poisoning was also studied as it appears to be antibiotic of choice in these parts. Agar diffusion assays were carried out using the Ap seeds N-hexane extracts. Gas chromatography and Mass spectroscopy of the extracts was also carried out to determine possible volatile bioactive compounds in the seeds. Studies on the alcoholic extracts have been done. Ap seeds extracts exerted a powerful anti-microbial effects in a dose dependent manner. On S. typhi, it showed 1.2-1.8cm zone of inhibition (zi) and on E. coli 1.5cm zi at 200mg/ml concentration, 31 compounds were discovered through GC/MS and included dodecane-1,7-dione a known antibacterial. The extracts exerted more antimicrobial effects than Metronidazole and compared favorably with Gentamicin. MIC of Ap seeds extracts on S. dysentariae was 0.8mg/ml. These findings suggest that Alligator pepper seeds hexane extracts offer good alternative as an antimicrobial agent for the treatment of food poisoning. Metronidazole should not be the drug of choice for food poisoning treatment and management. The study encourages further drug discovery work in order to confirm the particular bioactive compound/s in the N-hexane extracts responsible for the significant antimicrobial actions.
Session Title: AES11 - Microbiology of Food, including Spoilage, Fermentation and Probiotics: Antimicrobial Activity of Foods, Food Components, and Plant Endophytes
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 6719
Poster Board Number: SATURDAY - 869

Abstract Title:
Exposure to Manuka Honey Modules Antibiotic Susceptibility on Wound Isolates

Primary Author Block:
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Abstract Body:
Background: The clinical application of Manuka honey has recently gained momentum, particularly with regards to the treatment of chronic wound infections. Changes in antibiotic susceptibility have been observed previously, following the exposure of bacteria to subtherapeutic concentrations of honey, however such findings have been limited to Methicillin-resistant Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas aeruginosa. The aim of this study is to assess the modulation of antibiotic sensitivity in a broader panel of chronic wound isolates. Methods: Parent strains (P0) of Staphylococcus aureus, MRSA, S. epidermidis, Streptococcus pyogenes, P. aeruginosa, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis and Bacteroides fragilis were passaged ten times in the presence of sub-lethal concentrations of clinical grade Manuka honey to generate strain P10. In order to assess any permanent or transient changes in bacterial susceptibility, the bacteria were grown in honey-free media for a further 10 passages (X10). Antibiotic sensitivity testing was performed using a combination of microdilution and disc diffusion methodologies in order to determine MIC, MBC and MBEC values.

Results: Variable changes in bacterial susceptibilities were noted following subtherapeutic exposure to honey. P10 strains of S. aureus, S. epidermidis and S. pyogenes exhibited a ≥4-fold decrease in their sensitivities to erythromycin and tetracycline in comparison to baseline values. Similarly, P. aeruginosa displayed a 16-fold reduction in susceptibilities to both ciprofloxacin and gentamicin following passaging with honey. In contrast, K. pneumoniae and P. mirabilis showed notable increases in susceptibility towards both ciprofloxacin and gentamicin after 10 passages in the presence of honey. All changes in MIC, MBC and MBEC were shown transient in nature with the exception of P. aeruginosa (X10), which exhibited an MIC to ciprofloxacin >4 fold greater than the parent strain. Conclusion: Wound isolates exposed to clinical grade Manuka honey exhibited transient changes in antibiotic profiles. The underlying mechanism and clinical implications of such changes are unclear and warrant further investigation.
Antimicrobial Activities of Cranberry Extracts against Salmonella enterica Serovars from Broiler Chicken

Primary Author Block:
Q. Das1, D. Lepp1, K. Ross2, J. McCallum3, X. Yin1, K. Warriner4, M. Marcone4, M. Diarra1; 1AAFC, Guelph, ON, Canada, 2AAFC, Summerland, BC, Canada, 3AAFC, Charlottetown, PE, Canada, 4Univ. of Guelph, Guelph, ON, Canada

Abstract Body:
Background: Cranberry fruits possess antimicrobial properties due to its various acids and phenolics compounds, however, the underlying mechanism of actions are poorly understood. We evaluated the effects of cranberry extracts on growth rate and the transcriptome of Salmonella enterica serovars.

Methods: Two sub-fractions, anthocyanins (A20) and non-anthocyanin polyphenols (P85) from an ethanolic extract from cranberry pomaces (KCOH) were generated. The minimum inhibitory (MICs) and bactericidal (MBCs) concentrations of these fractions against S. enterica serovars Typhimurium, Enteritidis and Heidelberg were obtained using broth microdilution method (CAMHB) according to the CLSI’s guidelines. Transcriptional profiles of S. Enteritidis grown in cation adjusted Mueller Hinton broth (CAMHB) supplemented with or without 2 or 4 mg/ml of KCOH were compared by RNASeq performed on an Illumina MiSeq to reveal gene modulations serving as markers for biological activity. Results: The MIC and MBC of KCOH were 8 and 16 mg/ml, respectively against all tested S. enterica isolates, while these values were 8 and 4 mg/ml for A20 and P85 fractions, respectively. A20 and P85 induced up to 7 hours delay of growth initiation and a strong growth rate inhibition of Salmonella. Treatment of Enteritidis with KCOH revealed a concentration-dependent transcriptional signature. Exposure of Enteritidis at 2 mg/ml KCOH up-regulated expressions of 14 proteins, including phosphoenolpyruvate carboxylase, 4'-phosphopantetheinyl transferase (AcpT), pyruvate:ferredoxin (flavodoxin) oxidoreductase, multiple antibiotic resistance regulatory protein (MarB), Fe2+-enterobactin ABC transporter and SPI-2 type III secretion system effector (SpvB) and down-regulated expressions of 10 proteins. At 4 mg/ml of KCOH, expressions of 15 proteins including NarK family nitrate/nitrite MFS transporter were down-regulated while expressions of 5 proteins were up-regulated. Four genes were similarly modulated by both 2 and 4 mg/ml (two-component system response regulator DcuR, a membrane protein, a DUF91 domain-containing protein, and an anion permease) where anion permease down-regulated 6-fold at 4 mg/ml in comparison to control. Conclusion: Cranberry constituents affect growth and modulate expression of genes associated with metabolic functions, osmolality, iron uptake and nitrate/nitrite transport in Salmonella.
Abstract Title:
Ginger As An Inhibitor of the Multidrug Efflux Pump LmrS from Staphylococcus aureus and the Synergistic Effects of Ginger with Selected Antimicrobial Agents

Primary Author Block:
J. A. Adjei, L. M. Sanford, U. R. Cheeti, M. F. Varela; Eastern New Mexico Univ., Portales, NM

Abstract Body:
Staphylococcus aureus is an important pathogen of infectious disease. Multidrug-resistant strains such as methicillin-resistant S. aureus (MRSA) is resistant to many different antibiotics which makes this bacterium difficult to treat. The multidrug efflux pump LmrS from MRSA which confers resistance to the bacterium is known to actively extrude different antibiotics. To reverse antimicrobial resistance, inhibition of the multidrug efflux pump LmrS will be a good target candidate. Natural plant products are potential candidates for LmrS inhibition. The antimicrobial activity of ginger behind drug resistance inhibition is poorly understood. We explored the hypothesis that ginger extract inhibits the multidrug efflux pump LmrS and that ginger extract acts in synergy with selected antimicrobial agents. We found that ginger inhibited bacterial growth and LmrS ethidium bromide efflux. We conclude that ginger extract inhibits LmrS and works synergistically with selected antimicrobial agents.
Characterization of A Model Sys. Examining Fungal Endophytic Suppression of the Human Pathogen Enterohemorrhagic Escherichia coli in Spinacia Oleracea

Primary Author Block:
L. Gielda, S. Bates; Purdue Univ. Northwest, Westville, IN

Abstract Body:
Both bacterial and fungal endophytes reside in healthy tissue and play important roles in plant growth and pathogen defense. Beneficial fungal endophytes have been shown to inhibit the growth and spread of other microbes, such as plant pathogens, through competitive interactions including the production of secondary metabolites that influence microbial growth. Spinach and lettuce plants have been major sources of E. coli O157:H7 outbreaks, and the bacterium was recently shown to adopt an endophytic lifestyle within the tissue of these plants. In an effort to identify endophytes that hold the potential to suppress growth of E. coli O157:H7 in plantae, fungal endophytes in spinach were isolated and tested for their ability to inhibit bacterial growth. Of the endophytic fungi examined, four fungal isolates (PNW-2016-01 thru PNW-2016-04) demonstrated particularly strong bacterial growth suppressive properties and were identified based on DNA sequencing. The Stemphylium vesicarium isolate PNW-2016-03 limited the growth of a broad range of Gram-positive and Gram-negative bacteria, including E.coli O157:H7 in direct competition and indirect growth assays in vitro. While several Stemphylium species are known as plant pathogens, the development of a Stemphylium-spinach plant model system has shown the fungal endophyte positively influences the growth of spinach, suggesting a beneficial mutualistic relationship. Ongoing research is aimed at identifying specific bioactive compounds produced by Stemphylium PNW-2016-03 via chemical characterization including MS/MS-HPLC, and the bioactive mechanism of action through a bacterial transposon mutagenesis screen. Additionally, the endophyte-spinach model will identify influences of Stemphylium on community microbiome dynamics and plant health, as well as possible competitive inhibition of E. coli in plantae. As research related to the human microbiome has demonstrated a role for human associated microbes in contributing to personal heath and disease, this research examining the effects of resident endophytic microbes in plant health and microbial community dynamics could lead to the development of novel therapeutics with agricultural applications aimed at preventing the transmission of E. coli O157:H7 through produce consumption.
Abstract Title:
Exploration of Novel Lactic Acid Bacterial Strains from Plant Origins in Taiwan

Primary Author Block:
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Abstract Body:
Background: The lactic acid bacteria (LAB) industries in Taiwan with market size of over US$ 1 billion in 2016 have been developing for more than 50 years. The LAB comprises several phylogenetically closely related species which are generally recognized as safe (GRAS) probiotic strains, and are wildly used for the commercial fermented products, as well as applied in the feed industry for improve the performance in domestic livestock. As the only centralized microbial resource center in Taiwan, Bioresource Collection and Research Center (BCRC) is committed itself to provide reliable materials with accurate identification to meet users’ needs. We keep expanding the “local LAB bank” along with resource distribution, systematic identification, fermentation and downstream processing to assist LAB industries in Taiwan. Methods: 55 samples from plant origins including vegetable, fruit, silage and traditional fermented mustard products were collected. LAB were isolated with the dilution plate technique by MRS agar supplemented with 0.001% of both sodium azide and cycloheximide. Total of 128 LAB isolates obtained were identified by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) and 16S rRNA gene partial sequencing. Five novel strains were analyzed by two housekeeping genes, pheS and rpoA. Results: 128 LAB isolates can be discriminated in 22 groups based on MALDI-TOF profiling spectrum. The genera Lactobacillus and Leuconostoc were found in almost of all samples. The genera Lactococcus, Pediococcus, Weissella were occurred in few samples. According to phylogenetic analysis of 16S rRNA gene sequences, five strains belonging to the genus Lactobacillus obtained from fermented mustard products were classified as two possibly novel species groups. One group (3 strains) showed highest similarities to type strain of Lactobacillus paralimentarius (98.5%) and the other group (2 strains) showed highest similarities to type strain of Lactobacillus koreensis (98.4%). They showed low similarities of two housekeeping genes (pheS and rpoA) to the known type strains, less than 90% and 94.5%, respectively. Conclusions: The genera Lactobacillus and Leuconostoc were most abundant in fermented product samples. Comparative analyses of 16S rRNA, pheS and rpoA gene sequences demonstrated that the 5 novel strains were members of the genus Lactobacillus and classified as two possibly novel species groups. Further polyphasic approach is needed to classify accurately these two candidates into new taxa.
Abstract Title:
Utilization of Secondary Energy Sources by Selected Lactic Acid Bacteria, Candidates for Starter Or Adjunct Cultures for Commercial Cucumber Fermentations
Primary Author Block:
R. A. Ucar, I. M. Perez-Diaz; North Carolina State Univ., Raleigh, NC
Abstract Body:
Carbohydrate utilization in lactic acid bacteria (LAB), defines the extent of cucumber fermentations and the quality and long-term stability of the preserved fruit. Aside from glucose and fructose, secondary energy sources such as citrulline, trehalose, cellobiose, xylose, lyxose, gentiobiose, and furfural were previously detected in cucumber fermentation using metabolomics, prior to the undesire lactic acid degradation. The objective of the study was to evaluate the ability of candidates for starter or adjunct cultures for cucumber fermentations, including Lactobacillus plantarum, L. pentosus, L. brevis, L. buchneri and Pediococcus pentosaceous, to use secondary energy sources and aid in preventing spoilage. The natural content of the secondary energy sources in fresh cucumbers and commercial fermentations was corroborated using HPLC. The presence of putative substrates utilization and energy deriving pathways for the 7 compounds listed above were investigated using the publically available genome sequences and the KEGG Orthology and Integrated Microbial Genomes tools. The influence of oxygen availability and pH on metabolism of the targeted compounds was studied using a fermented cucumber juice model system. Conversion of metabolic substrates was monitored using HPLC. While the presence of gentiobiose, cellobiose and lyxose was unconfirmed in fresh cucumber juice and fermentation cover brines collected on day 3 of commercial fermentations, trehalose and xylose were sporadically detected to 15.5 ± 1.6 and 36 mM, respectively. The ability of L. plantarum and L. pentosus to convert cellobiose into glucose and trehalose via the starch & sucrose pathway, but not xylose was confirmed. While the putative arginine biosynthesis pathway was found in all the targeted genome sequences, L. buchneri was unique in converting citrulline to arginine. Inconclusive results were obtained with regards to furfural utilization. Significant changes in the ability to utilize the substrates of interest were observed as a function of pH, yet no substantial differences were observed in response to oxygen availability. The results suggest the tested LAB are able to selectively utilize secondary energy sources in fermented cucumber juice at variable rates, possibly enabling their combined and strategic utilization as starter or adjunct cultures.
Background: Ogi” is a fermented cereal gruel prepared from maize (Zea mays), millet (Pennisetum typhoideum) or guinea corn (Sorghum bicolor) (Adeyemi, 1993). It could be boiled to give a thicker consistency wrapped in leaf allowed to cool and set to a gel known as “eko” or “agidi” (Adeyemi, 1983) and it is often marketed as a wet cake wrapped in leaf or transparent polythene bags. This research estimated the populations and profiles of moulds species present in Ogi and Eko after spoilage sets in.

Methods: Molds were isolated on Sabouraud Dextrose Agar [SDA] incubated at 28°C±2°C for 72h and identified by standard microbiological methods. Screening of moulds for amylase activity was determined on starch agar medium and flooded with gram’s iodine. Amylase production was carried out on selected six mould isolates using solid state fermentation using rice bran as the medium. The crude enzyme was recovered using phosphate buffer of pH 6.5. Studies were carried out on the alpha amylase and glucoamylase activity using the DNS method. Results: Mean mould population observed on the spoilt food samples ranged from 5.06 log cfu/g for ogi to 5.80 log cfu/g for eko wrapped in leaves. Thirty three (33) moulds strains were isolated during spoilage of Ogi and Eko and this include A. niger, A. flavus, A. fumigatus, Rhizopus sp. and Penicillium sp. Out of the thirty three (33) isolates screened only twenty one (63.3%) were found to be amylase positive by showing a clear zone around their colonies after flooding with iodine solution while negative organisms shows a blue black colouration around their colony. Aspergillus niger isolated from spoilt Eko wrapped in leaf has the highest percentage (33.4 %) alpha amylase activity and Aspergillus flavus isolated from spoilt raw Ogi has the lowest percentage alpha amylase activity (18.2%). Aspergillus niger isolated from spoilt Eko wrapped in nylon produces the highest glucoamylase activity (210 U/ml) as evident while Penicillium sp. isolated from spoilt cooked Ogi had the lowest glucoamylase activity (80U/ml) Conclusions: This study has been able to identify naturally occurring fungal isolates that can be used in producing amylase and provides additional information to support research in future years about microbial enzymes production potential of these fungal isolates. However further research is needed to optimized the activities of this enzyme to make industrial scale.
Session Number: 253
Session Type: Poster
Session Number: 253
Session Type: Poster
Session Title: AES11 - Microbiology of Food, including Spoilage, Fermentation and Probiotics: Mechanisms and Control of Food Spoilage
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 4529
Poster Board Number: SATURDAY - 877

Abstract Title:
Bacteriology and Chemical Quality of Fresh and Wood - Smoked Catfish from Feral and Cultured Aquatic Habitats of the Niger Delta, Nigeria

Primary Author Block:
G. E. Udofia1, D. I. Ikpe2, E. S. Ntino1, O. D. Akan3; 1Univ. of Uyo, Uyo, Nigeria, 2Akwa Ibom State Polytechnic, Ikot Ekpene, Nigeria, 3Akwa Ibom State Univ., Oruk Anam, Nigeria

Abstract Body:
Bacteria load and chemical burden including total petroleum hydrocarbon (TPH), total hydrocarbon content (THC) and polycyclic aromatic hydrocarbons (PAHs) in water, fresh and wood- smoked catfish from the wild and cultured aquatic systems were investigated using standard procedures. The results of the bacteria load revealed a higher mean value of $2.1 \times 10^6$ CFUg$^{-1}$ and $2.2 \times 10^3$ CFUg$^{-1}$ in tissues of fresh and smoked fishes from the natural (feral) water, respectively; and a lower mean value of $3.1 \times 10^3$ CFUg$^{-1}$ and $1.9 \times 10^2$ CFUg$^{-1}$ in fresh and smoked fishes from cultured system, respectively. Amongst the sixteen genera isolated from samples from feral habitat, Bacillus, Pseudomonas, Escherichia, Salmonella and Enterococcus were dominant with 13.97 %, 11.76 %, 11.76 %, 11.03 % and 8.82 % distribution, respectively. The dominate bacterial species from cultured habitat were Salmonella (13.1 %), followed by Bacillus (11.5 %) and Pseudomonas (11.5 %), while the least species were Vibrio and Clostridium with (3.3 %) each. The TPH and TPC of 5.24 mgl$^{-1}$ and 3.01 mgl$^{-1}$ were higher in natural water when compared to 0.13 mgl$^{-1}$ and below detectable limit in pond water. Also the catfish from the natural water body bioaccumulated higher concentrations of these substances than those from the pond, (2.15 µgkg$^{-1}$ and 0.43 µgkg$^{-1}$ for catfish from wild waters and 0.024 µgkg$^{-1}$ and below detectable level in fish samples from pond). The presence and concentration of ten PAHs suit were detected in fish samples with wood-smoked fishes containing 1.592 µgkg$^{-1}$ and 0.899µgkg$^{-1}$ for wild and pond fishes respectively. The ratio of phenanthrene to anthracene (1.08) in smoked cultured fish suggests that the PAHs are from combustion sources. Similarly the ratio of fluoranthene to pyrene (1.58) in smoked fish (feral fish) suggests that the fish is contaminated with PAHs from pyrolytic origin. It could therefore be inferred that wood smoking as a method of preserving fish could enhance the qualitative and quantitative PAHs status in smoked products.
Quorum Sensing AsaI Mutants Affect Spoilage Phenotypes, Motility, and Biofilm Formation in a Marine Fish Isolate of Aeromonas Salmonicida

Abstract Title:
Quorum Sensing AsaI Mutants Affect Spoilage Phenotypes, Motility, and Biofilm Formation in a Marine Fish Isolate of Aeromonas Salmonicida

Primary Author Block:
L. Liu, Y. Yang, L. Feng, J. Zhu; Zhejiang Gongshang Univ., Hangzhou, China

Abstract Body:
Aeromonas salmonicida has also been identified as part of spoilage microbiota in ice-stored fish. Microbial spoilage is associated with the regulation of quorum sensing (QS). An A. salmonicida AE03 with QS mediated acylated homoserine lactones (AHLs) activity was isolated from spoiled large yellow croaker (Pseudosciaena crocea). In this study the activity and role of AHLs in spoilage phenotypes, motility and biofilm formation of AE03 were investigated. The strain AE03 could induce Chromobacterium violaceum CV026 to produce the purple response both at 28 °C and 4 °C, exhibiting a density-dependence. Five types of AHLs were detected in AE03 culture by LC-MS/MS analysis, and N-butanoyl-L-homoserine lactone (C4-HSL) was a major signal molecule, reaching the highest activity at 28 °C for 30 h. An asaI-mutant, constructed by a suicide plasmid, failed to produce short chain AHLs signal. Compared with wild type (WT) strain, the production of trimethylamine (TMA), biogenic amino and protease significantly increased in asaI-mutant during the exponential and stationary phase, while the growth rate didn’t differ. Swimming motility in asaI-mutant was comparatively stronger than that of WT strain, whereas, asaI-mutant resulted in the decrease of maturing biofilm. Furthermore, supplementation of exogenous C4-HSL restored the production of spoilage metabolites, protease and biofilm formation in mutant. In accordance with the effect of asaI deletion on the spoilage phenotypes and motility, asaI-mutant was showed to significantly up-regulate the transcript levels of torA, cadA and fliR, as well as asaR, indicating that C4-HSL could be involved in the modulation of the spoilage related enzymes and flagella. Indeed, the asaI-mutant promoted the spoilage progress of fish juice stored at 4 °C, while exogenous C4-HSL repressed the TVB-N accumulation. The present study highlighted that the AHL synthase/AsaI was an important regulator in spoilage, motility and biofilm formation of A. salmonicida, and spoilage potential was under the negative control of AsaI/AsaR-type system.
Abstract Title:
Shelf-Life Study of Processed and Packaged Cocoyam Flour During Storage At Room Temperature 29.0 ±2OC

Primary Author Block:
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Abstract Body:
Nigeria produces the largest amount of cocoyam in the world (5million mt). The tuber is subject to microbial spoilage during post harvest. Food and feed contamination with pathogenic and toxigenic microorganisms is of current concern and has received a great deal of attention during the last three decades. The shelf life study of dry milled and packaged cocoyam was carried out for a period of four months. 3000g of the tuber were purchased in Ekpoma market, in Nigeria, processed into powdered form and packaged (100g per pack) in low density polyethylene bags. Microbiological and physicochemical qualities of the packaged samples were carried out using standard microbiological and chemical methods while nutritional analyses was done according to the official methods of AOAC. Aflatoxin detection was done using ELISA. There was gradual increase in the bacterial counts during the storage period. The total viable bacterial count ranged from 1.6x10³ - 4.8x10⁵ cfu/g during the period of study. The bacteria isolated include Bacillus subtilis, Proteus species, Staphylococcus epidermidis, Micrococcus species, Klebsiella species, Staphylococcus aureus. The total viable fungal count increased from 5.0 x 10¹ - 3.8 x 10⁵ cfu/g during the period of study. A total of four (4) fungal genera were isolated from the packaged cocoyam flour, they include; Penicillium species, Aspergillus flavus, Fusarium species and Rhizopus species. The pH of the samples decreased during storage from 6.40 ± 0.02 to 4.17 ± 0.01 while the %Titratable acidity increased from 0.024 ± 0.002 to 1.116± 0.02 at 0 hr and 4th month respectively. Aflatoxin B1 and B2 content at the end of storage were 0.097, 0.063 µg/Kg respectively. There was an increase in % moisture content and slight decreases in % carbohydrate, protein and crude fibre after 4 months of storage. The concentration of the aflatoxin determined were found to be within the acceptable limits set by Nigeria's National Agency for Food and Drug Administration (NAFDAC) of 4 µg/Kg for cooked/ready-to-eat food. The condition under which the cocoyam flour was processed is therefore recommended for the commercial production of the food item.
Abstract Title:
Bio-Protective Lactic Acid Bacteria Cultures that Inhibit Lactobacillus Wasatchensis

Primary Author Block:
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Abstract Body:
Late gas defects in aging cheese result in significant losses to the manufacturer. Lactobacillus wasatchensis, a new non-starter lactic acid bacterium (NSLAB), was recently identified as an important cause of late gas defect. Controlling growth of this unwanted NSLAB may be possible by incorporating bio-protective lactic acid bacteria (BP-LAB) cultures into the cheese during manufacture, which would inhibit Lb. wasatchensis growth during cheese aging. Previous research has shown several BP-LAB cultures inhibit Lb. wasatchensis to varying degrees but extent and mode of inhibition were not determined. In addition, other potential BP-LAB strains were also tested for their inhibitory capacity.

Quantification of inhibition between BP-LAB cultures and Lb. wasatchensis was done using the spot test with the agar-flip method then measuring inhibition zones over time. MRS agar with 1% ribose (MRS-R) was inoculated with each BP-LAB and incubated anaerobically at 35°C for 48 h to form a spot colony. Inoculated agar was flipped over and a Lb. wasatchensis strain swabbed on the exposed surface then plates were incubated anaerobically at 25°C for up to 72 h. The five most inhibitory BP-LAB cultures were Lactobacillus rhamnosus LB3, Lactobacillus paracasei P-210, Lactobacillus brevis ATCC 13648, Lactobacillus casei F19, and Lactobacillus paracasei LILA. In addition, potential synergistic quantification of inhibition by co-BP-LAB strains was tested by mixing 1 mL each of two different BP-LAB strains, and then repeating the agar-flip protocol. Four co-cultures were tested LB3/ P-210, LB3/P-220, P-200/P-210, and P-200/P-220. No significant increases in inhibition zones were observed when BP-LAB cultures were paired versus individual strains. Results confirm selected BP-LAB strains can inhibit growth of Lb. wasatchensis. Initial results also suggest some BP-LAB cultures may be producing bacteriocins that inhibit Lb. wasatchensis. Addition of selected BP-LAB cultures during cheesemaking could control late gas defect during cheese aging.
Abstract Title:
Effect of Organic Acids on Suppressing Growth of Lactobacillus Wasatchensis Wdc04

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Abstract Body:
Lactobacillus wasatchensis is a slow growing, non-starter lactic acid bacterium (NSLAB) causing late gas formation in aging cheese, which results in significant economic losses to the producer. During cheese aging, organic acids can be produced by other NSLAB cultures or purposefully added to cheese during manufacture. Organic acids are often used as food preservatives, can occur naturally in foods, generally don’t affect flavor or product quality, and, under acidic conditions, enter bacterial cells altering the cell’s proton motive force. Selected organic acids, in their naturally occurring concentration ranges in Cheddar cheese, were investigated for their ability to inhibit Lb. wasatchensis. Five organic acids (lactic, formic, propionic, citric, and acetic) produced by NSLAB organisms were tested. They were each added at their minimum, median, and maximum concentrations, as found naturally in aged Cheddar cheese, to individual wells of a 48 well plate containing MRS broth with 1% ribose (MRS + R) inoculated with Lb. wasatchensis WDC04. Growth rates were determined on a Tecan Infinite 200 PRO spectrophotometer over 40 hours with results graphed on Excel. Initially, tests were done at pH 7.0 with several acids exhibiting some inhibition. Tests were then run at pH 5.0 to determine if the organic acids were more effective at a pH of aged cheese. Both formic and citric acid showed significant inhibition of Lb. wasatchensis WDC04. Formic acid was the most inhibitory of all five organic acids with the maximum concentration (100 mM) showing the greatest inhibition. Addition of citric acid at the minimum (12 mM) and median (13.5 mM) concentrations also produced inhibition. Use of selected organic acids at concentrations normally found in Cheddar cheese could be a potential antimicrobial measure to prevent or reduce late blowing in aging cheese.
Abstract Title:
Kinetics of Spoilage Bacterial Growth in A Simulated Vacuum-Package Beef Environment

Primary Author Block:
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Abstract Body:
Spoilage of meat, signified by off-odours, off-flavours, or discolouration, is caused by microbial growth and biochemical changes in beef. Intrinsic (pH, glucose and lactic acid concentration) and extrinsic (temperature and atmospheric condition) factors influence the growth of spoilage bacteria on beef. A quantitative description of microbial community formation under different physico-chemical conditions can inform the development of shelf-life predictive models, by defining factors responsible for the development of bacterial spoilage community structure, and thus informing strategies to extend the shelf-life of commodities such as vacuum-packaged (VP) beef. In this study, a prototype community of three bacterial species (Carnobacterium maltaromaticum, Brochothrix thermosphacta, Serratia liquefaciens) derived from VP beef samples was used to investigate the effects of pH, lactic acid and glucose on bacterial growth kinetics, within a VP beef model system. Eight different growth media were formulated combining two levels each of pH, lactic acid and glucose in a brain heart infusion broth base. Formulated media were transferred into commercial VP bags, vacuum-sealed (99% vacuum) and then heat-shrunk. An inoculum of individual strains was injected into bags of media through a sterilized septum, and the bags incubated at 100°C. Samples were removed via septum ports at each time interval, serially diluted, and plated on BHI agar. Two trials were performed for each strain and medium formulation. DMFit and SAS software were used to calculate growth rates and data analysis, respectively. Glucose did not have a significant effect on growth rate (p >0.05), whereas pH and lactic acid were significant factors (p <0.0001) for each of the species. Additionally, undissociated lactic acid, resultant of pH and lactic acid, showed equal significance (p <0.0001) on growth rate. A similar trend for maximum population density was observed for all three species. Based on these results, we conclude that lactic acid and pH are significant factors that potentially shape beef spoilage communities in a VP environment. The findings provide the foundation for subsequent studies to quantitatively describe interactions among mixed populations of bacteria that predominate on VP beef, and to better understand the effect on shelf-life.
Effects of Electron Beam Irradiation on Microbial Quality of Pork Products

Primary Author Block:

Abstract Body:
The US FDA approved irradiation for red meats and poultry to control food-borne pathogens and extend the shelf-life of products. Electron Beams (EB) are thought to offer several advantages over gamma irradiation. In view of this, the scientific study was undertaken to assess microbiological quality of EB irradiated pork products to determine the shelf-life henceforth provide safe products to consumer. Freshly prepared ready-to-eat pork products such as pork sausages and salami were procured from HACCP & ISO certified processing plants. Each containing 100 g of product, heat sealed in LDPE pouches and taken to Board of Radiation and Isotope Technology, Navi Mumbai, India and exposed to 3.0, 3.5 and 4.5 KGY doses of EB irradiation and stored at refrigeration temperature (0-4°C). Microbiological analysis of irradiated pork products was carried out using TVC, Selective and differential media used for enumeration of Staphylococcus, E. coli, Salmonella spp., Listeria spp., Bacillus cereus, Psuedomonas spp, yeast and mold count. All the irradiated and control pork products stored at refrigeration temperature were analysed for the determination of microbiological quality at different time interval to know its shelf-life. The average TVC (log cfu/g) for the control pork sausage and salami samples on 0 day was found to be 5.59±0.12 and 5.85±0.03 respectively, whereas, the average TVC (log cfu/g) on 0 day for irradiated pork sausages and salami with dose rate of 3.0, 3.5 and 4.5 KGY were observed as 4.48±0.14, 4.06±0.10, 4.00±0.03 and 3.95±0.10, respectively. The study indicated that there was significant (p≤0.05) reduction in microbiological load in pork products with increasing irradiation dose. Inhibitory effect of EB irradiation was observed on E. coli and Salmonella spp. count throughout the storage period in the irradiated samples. However, none of the irradiated and control pork product samples were found to contain B. cereus, Pseudomonas spp. and Listeria spp. throughout the entire storage. Thus, the shelf-life of control and irradiated pork sausage with 3.0, 3.5 and 4.5 KGY doses was found to be 6, 12, 15 and 27 days, respectively. Whereas, the respective shelf-life of control and irradiated pork salami samples was found to be 6, 21, 23 and 29 days at refrigeration temperature. The study concluded that EB irradiation at the dose rate of 4.5 kGY was found to be more effective in reducing the microbiological load and extension of shelf-life of pork sausage and salami at refrigeration storage up to 27 and 29 days, respectively.
Abstract Title:
Application of Atmospheric Cold Plasma-Activated Water (Paw) Ice for Preservation of Shrimps (Metapenaeus Ensis)
Primary Author Block:
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Abstract Body:
Background: Cold plasma (also called non-thermal plasma), has been a novel non-thermal sterilization technology in food industry. Various reactive species produced in the cold plasma, such as free radicals (e.g. ROS/RNS), ions and ultraviolet (UV) photons, are thought to be responsible for efficient inactivation of microbes. Many previous studies have demonstrated the strong bactericidal effect of cold plasma on microbes in food products. Due to its low temperature properties, strong bactericidal capacity and nontoxic byproducts, cold plasma has prospect of wide application in food industry. Recently, cold plasma-activated water (PAW) as a disinfectant, has been proposed for microbial control of fresh food products. However, there are only limited studies concerning about the application of cold plasma for the preservation of aquatic food. Considering that seafood requires for low temperature storage, PAW ice is proposed for the first time in this study in order to apply for preservation of seafood. Methods: Microbiological analysis; pH determination; Total volatile basic nitrogen (TVBN) measurement; Firmness determination; Color evaluation; Ca2+–ATPase activity determination; SDS-PAGE assay; Total sulfhydryl group (-SH) determination; Surface hydrophobicity. Results: Compared with TW ice, PAW ice has huge potential to inhibit microbial growth in the shrimps, prolonging the acceptable period from 4 to 8 days. The pH of PAW ice treated shrimps remained below 7.7 during storage period. The obvious bad changes in color characteristics and hardness have been delayed with PAW ice treatment. The production of volatile basic nitrogen (TVBN) was retarded below 20 mg/100g during PAW ice storage, significantly lower (p < 0.05) than that of TW ice treated group. PAW ice did not result in adverse effects on shrimp proteins. Conclusions: In this study, the PAW ice was shown to have the ability to inhibit the rapid growth of microorganisms, and retard the loss of shrimp quality and freshness, resulting in prolonging the shelf-life. PAW could be considered as a potential alternative method for preservation of food products in the future. However, the precise control of reactive species in PAW ice requires the development of equipment modification and the safety of PAW ice should be further evaluated in the future.
Identification of Novel Immunomodulatory Probiotic Strains Isolated from Pulque

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Abstract Body:
Probiotics are live, nonpathogenic microorganisms that confer benefits to human health when administered in adequate amounts. Currently, there is a great interest in the probiotic potential (in particular anti-inflammatory and anti-cancer effects) of lactic acid bacteria (LAB) isolated from nonconventional sources, such as traditional fermented foods. Pulque is a traditional Mexican beverage and LAB is one of the most predominant groups inside. In this study we evaluated both the anti-inflammatory and anti-cancer properties of different LAB isolated from pulque. A total of 14 strains were isolated, species identification was performed by 16S rRNA. These strains were then tested for their ability to block the production of IL-8 (a pro-inflammatory cytokine) in HT-29 cells stimulated with TNF-α. Bacterial strains able to block IL-8 production (and thus classified as anti-inflammatory) were tested in vivo in a DiNitro-BenzeneSulfonic-acid (DNBS)-induced chronic colitis mouse model, weight loss, gut permeability and cytokine profiles were used as readouts of inflammation. In parallel, sulforhodamine B assays were performed to evaluate the anti-proliferative effects of different lactobacilli isolates on HT-29 cells, apoptosis by Annexin V staining, as well as the possible mechanisms of anti-cancer properties by qPCR analyses (Casp8, Casp9, ErbB2 and ErbB3 genes expression). The isolates belonged to L. brevis, L. plantarum, L. paracasei, L. composti, and L. sanfranciscensis. L. plantarum LBH1064, L. sanfranciscensis LBH1068 and L. composti LBH1072 significantly block IL-8 production. Strikingly, L. sanfranciscensis LBH1068, improved health in enflamed mice as observed by a reduction of weight loss, significant decreases in gut permeability, and cytokine modulation. On other hand, L. brevis LBH1073 was able to suppress 40% of proliferation of HT-29 cells as the positive control 5-FU (a drug used in cancer treatment). In addition, this strain was also able to increase 25% the amounts of annexin V+/PI+ (late apoptotic cells). Finally, L. brevis LBH1073 promotes apoptotic cell death (one of the major anti-tumor therapeutic direction of new treatment) via Casp8 and Casp9 (starter genes in TNF-α apoptosis pathway) activation and suppressing expression of anti-apoptotic genes ErbB2 and ErbB3. Altogether, our results highlighted the potential of lactobacilli isolated from pulque and in particular L. sanfranciscensis LBH1068 and L. brevis LBH1073 as novel probiotic strains to treat IBD and cancer, respectively.
Abstract Title:
Exploring the Potential of Lactic Acid Bacteria Isolated from Gastrointestinal Tracts of Poultry for Invitro Probiotic Traits

Primary Author Block:
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Abstract Body:
Background: Lactic Acid bacteria (LAB) are one of the safest group of bacteria recognized as safe and of probiotic potential. These bacteria makeup for the highest proportion of the microorganism present in the gastrointestinal tract of human and animals. The efficacy of locally isolated LAB from poultry intestinal samples were evaluated for their invitro probiotic potentials. Methods: Different parts of the intestinal tract of 16 birds, including the crop, small intestine, large intestine and cecum were collected in sterile conditions and processed for the presence of LAB. Appropriate serial dilution of the samples were plated onto DeMan, Rogosa and Sharpe (MRS) agar, MRS supplemented with 0.02% bromocresol purple, and Blood agar plates. Gram-positive, non-hemolytic, catalase negative, non-motile and non-spore formers were presumed to be LAB and selected for probiotic characterizations. Acid and bile tolerances at different time intervals, antibacterial spectrum against pathogens, survival rate in simulated gastric and intestinal conditions, and antibiotic sensitivity profile of the selected isolates were evaluated. All isolates were screened for their auto-aggregation, co-aggregation, hydrophobicity and adhesion to Caco 2 cell lines. The selected LAB isolates were identified using a pair of primer Lacb (Forward): 5’-TGCCTAAATACATGCAAGT-3’ and Lacb (Reverse): 5’-CTTGTTACGACTTCACC-3’. The amplified products were sequenced and the obtained sequences were aligned to 16S rRNA gene sequences in the GenBank data base using the BLAST algorithm. Results: A total of 78 colonies isolated from broiler chicken intestinal samples presumed to be LAB were tested for their probiotic characters. The isolates indicated variable level of acid and bile tolerance, while only 17 isolates survived the simulated gastric conditions. Among these isolates, 9 isolates demonstrated inhibitory actions towards Salmonella typhimurium and S.enteriditis and showed desirable antibiotic sensitivity profile. The autoaggregation, co-aggregation and hydrophobicity percentages of the isolates were variable and only five of the isolates appeared significantly adhesive to Caco2 cell lines. Based on 16SrRNA sequencing, the isolates were identified as Lactobacillus plantarum, (2), L.fermentum, Pediococcus acidilactici and Enterococcus faecium. Conclusions: Five of the LAB isolates harbored significant probiotic traits and were selected for invivo evaluations to prove their probiotic effects in the respective host.
Abstract Title:
Autolyse the Cell in Order to Save It? Triggering Autolysis As A Strategy for Improving the Viability of L. Reuteri

Primary Author Block:
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Abstract Body:
From promoting wound healing and increasing the production of Vitamin D to lowering cholesterol levels, L. reuteri has the potential to impart a variety of health benefits. However the viability of beneficial bacteria cannot be maintained in probiotic food products during storage. The objective of this study was to examine whether choline and its derivative hemicholinium-3 (HC-3) can be used to preserve viable cells of L. reuteri in autolytic models. A phosphate-induced autolytic model in de Man, Rogosa and Sharpe media (MRS) was used. Cell lysis was determined by measuring the turbidity of cultures by determining optical densities at 610 nm. Viable cell counts were determined by plating on MRS-agar following by the counting of colony forming units. Choline and hemicholinium-3 (HC-3) significantly blocked autolysis of L. reuteri at 360 mM and 4 mM, respectively. Viable cell counts corroborated these observations. Importantly, autolytically induced cells treated with choline and hemicholinium-3 were significantly more viable than even non-induced cells (whether treated or untreated). Over-production of the autolytic protein spirosin was noted during induction of autolysis. Over-production of this protein was not attenuated with choline and HC-3. This result indicated, that while choline and its derivatives blocked the effects of autolysis, these reagents did not block the signaling processes that lead to autolysis. In conclusion, inducing autolysis, and then blocking it with choline and its analogs, is a promising approach for retaining the viability of L. reuteri cells.
Abstract Title:
Impact of Fructooligosaccharides (Fos) & Inulin on the Survival and Growth of Lactobacilli

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Abstract Body:
Background: Consumption of fermented foods facilitates the digestion by retaining an accurate balance of bacteria in the intestine, thus improving the whole immune system. Due to this healthiness, universal fermented foodstuff markets are at a mounting demand. Fermentation of lactic acid plays the role of protagonist in functional foods and preserving commodities, fruits and vegetables for human consumption worldwide. The target site of action of lactobacilli is the intestine, where they ferment food-derived indigestible carbohydrates like inulin and FOS. Mechanistically fermented foods causes an increase in gut bacterial content, which is beneficial for nutrition and human health. Therefore, they must be surviving the gastrointestinal acidic condition before reaching the target site. It is essential to maintain the effectiveness and safety profile food preparations to stimulate the growth. Methods: The present study aims to encapsulate Lactobacillus plantarum(6161) with inulin and FOS to keep maximum effectiveness. Microencapsulation via extrusion technique was used for protection from gastric acid stress and its target delivery. The formulation F1 (Inulin/LP/ALG) and F2 (FOS/LP/ALG) were made with different concentrations FOS and Inulin 0%, 2% and 3% (w/v) in 2% (w/v) alginate solution. Both the formulations were tested against Artificial Gastric Juice (AGJ) and Artificial Intestinal Juice (AIJ). Results: As a result, the formulation with 3% (w/v) inulin and 2% FOS was found most effective and survived the purported hostile conditions. F1 has shown greater viability 9 log CFU/mL in contrary to F2 8 log CFU/mL after 2hr incubation in artificial gastric juice (AGJ) at pH 2.0 and reduced viability was observed at 2% bile (AIJ) after 2hr. In stability study, after one month storage at 4°C approximately 9 log CFU/mL viability was observed. Conclusions: In conclusion, use of inulin and FOS modulated the growth of Lactobacillus plantarum and kept the strain viable in all antagonistic conditions. Hence, the consumption of lactobacilli formulations may have predominant impact in improvising inflamed gut health, amplifying the immune system, lactose intolerance, reducing the prevalence of allergy, dementia, Chronic fatigue syndrome (CFS), Non-alcoholic fatty liver disease (NAFLD) and risk of certain cancers.
Label Claim Analysis of Commercially Available Probiotic Yogurts in Pakistan

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Fermented dairy product usage has a long history in human culture because of their many beneficial effects. Many of these products are available in the markets with various claims. Worldwide considerable research is taking place for further understanding the science of regulating probiotic foods; however there is still a death of literature on the subject in Pakistan. This study involved evaluating commercial yogurt brands available in major urban centers of central and western Pakistan. The products were tested for accuracy of content claim by determining their total viable count. Samples were cultured on 4 different media i.e. MRS for anaerobic growth whereas M17 and nutrient agar for aerobic growth and MacConkey agar for coliform count. Viable bacterial counts showed bacterial loads ranging from 10^6 to 10^9 CFU g^-1. The results do not show clear compliance with international standards as according to Codex Alimentarius (2003), a minimum of 10^7 CFU g^-1 for starter cultures and a minimum of 10^6 CFUg^-1 for added probiotics is recommended. Out of total 63 isolates from 24 samples, 8 were reported gram negative and 55 were gram positive (7 cocci and 48 rods). Genus and species specific PCR was performed using five different primers for lactobacillus, L. delbrueckii, S. thermophilus, bifidobacteria and L. lactis. PCR confirmed the absence of bifidobacteria and S. thermophilus in any of the samples whereas many lactobacillus and L. delbrueckii and few L. lactis were identified. Further Probiotic potential characterization on identified isolates showed few isolates tolerant to acidic pH and most of them were tolerant to bile salt concentrations. While two isolates showed significant auto-aggregation property. Furthermore, all of the pH, bile tolerant and auto-aggregating strains were L. delbrueckii positives confirmed through PCR. This study highlights the need for developing science based guidelines for accurate labeling and manufacturing of products. Establishment of post market surveillance mechanism is also needed for ensuring that an efficacious and safe product reaches to the local consumer.
Abstract Title:
Rapid Method for Measuring the Effect of Prebiotics on Probiotic Bacteria Growth

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Abstract Body:
Prebiotics are used to stimulate probiotic bacterial growth in the gut to optimize their health benefits. A rapid method was developed to evaluate potential growth enhancement by prebiotics on probiotic bacteria using a programmable spectrophotometer, standard microtiter plates, and commercial media, with growth enhancement results ready in 12 hours. Lactobacillus strains were grown in MRS broth while Bifidobacterium strains were grown in MRS broth with L-cysteine. Each culture was back diluted to an OD600 of 0.1 with the appropriate MRS broth then inoculated into wells (48 well plate) containing individual prebiotics. Plates were placed in a Tecan Infinite M200 spectrophotometer and incubated at 37°C with A600 readings taken for 12 h. Growth curves were done in triplicate with results compared to controls to determine extent of prebiotic growth enhancement. To optimize the method MRS concentrations of 20%, 35%, 50% and 100% were tested at selected pHs (7.0, 5.5, 5.0, 4.5, and 4.0) using 5 probiotic cultures. Addition of the bio-catalytic oxygen-reducing reagent oxyrase to test wells just prior to testing significantly enhanced growth of Bifidobacterium species and some lactobacilli such as Lb. acidophilus. Results indicated a 25% MRS broth at pH 5.0 with 2% oxyrase addition optimized prebiotic growth enhancement. Using this method, the stimulatory effect of added prebiotics (2% v/v) FOS, GOS, and XOS was determined for Bifidobacterium infantis M-63, Bifidobacterium longum BB536, and Bifidobacterium lactis BL-04, Lactobacillus rhamnosus LR-32 and Lactobacillus acidophilus NCFM. All three significantly improved growth of M-63, but only FOS increased growth of BL-04. For BB536, just GOS enhanced growth. GOS and FOS slightly improved growth of NCFM but no oligosaccharides enhanced growth of LR-32. The method allows rapid testing of various inoculum levels, prebiotic concentrations, media pHs, and prebiotic combinations for any probiotic strain including Bifidobacterium. With multiple samples run concurrently, comparisons can readily be made. In addition, the method can determine optimum enhancement of individual prebiotics or prebiotic combinations for any probiotic strain. 1
Yeast Cell Wall Mannan-Rich Fraction Reduces the Prevalence of Antibiotic Resistant Escherichia coli in Broiler Chickens

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Abstract Body:
Background: The emergence of bacteria that are resistant to antibiotics is causing great concern globally, with estimates indicating that by 2050 the issue of antibiotic resistance could cost $100 trillion (O'Neill, 2014). Within the agricultural industry, the requirements for profitability and the providence of the world’s food supply has driven the widespread use of antibiotics as growth promoters. An acceleration in the prevalence of antibiotic resistance has mounted pressure for changes in the control, increased monitoring and cessation of antibiotic use as growth promoting agents. These developments illustrate the necessity to find alternative strategies to the use of antibiotics, which improve animal health without generating drug resistant bacteria. The primary focus of this research is to assess the effect that dietary supplementation with mannan rich fraction (MRF) from the cell wall of Saccharomyces cerevisiae has on mitigating population levels of antibiotic resistant Escherichia coli.

Methods: The ability of MRF to reduce the prevalence of antibiotic resistance was assessed through isolation of Escherichia coli from the caecal contents of day 35 post hatch broilers whose diet was supplemented with MRF or control broilers not supplemented with MRF. MRF supplementation was included in the diet in a step down program; Starter - 1300g/t (Day 0-10), Grower - 1000g/t, (Day 11-25), Finisher - 600g/t (Day 26-35). Antibiotic susceptibility testing of these isolates was carried out at sensitive, intermediate and resistant break point levels using replica plating in the presence and absence of the antibiotics; ampicillin, tetracycline, doxycycline, piperacillin and ticarcillin.

Results: The supplementation of MRF in the diet of broilers showed a decrease in the number of antibiotic resistant isolates in their caecal contents compared to those fed the basal control diet. The population levels of ampicillin, tetracycline, doxycycline, ticarcillin and piperacillin resistant E. coli were decreased by 79%, 87%, 92%, 80% and 70% respectively. Conclusion: Our results suggest the positive effect that dietary supplementation with MRF has on the prevalence of antibiotic resistant Escherichia coli and represents a potential strategy to mitigate antibiotic resistance.
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Session Title: AES11 - Microbiology of Food, including Spoilage, Fermentation and Probiotics: Probiotics, Prebiotics and Feed Supplements
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 6276
Poster Board Number: SATURDAY - 892

Abstract Title:
Monitoring Uniformity of Bifidobacterium Animalis Subsp. Lactis Hn019™ and Bl-04™ and Lactobacillus Rhamnosus Hn001™ over the Production of A Probiotic Dietary Supplement Blend Using Strain-Specific Droplet Digital Pcr

Primary Author Block:
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Abstract Body:
The probiotic industry is seeing an increase in requests for multi-strain probiotic dietary supplements. This is due to consumers are becoming more educated on probiotics and are requiring high quality dietary supplements that meet the structure/function claims being made by the manufacturers. Structure/function claims allows statements to be made associating to the body’s normal structure or function, as proven by human clinical trials, which study particular probiotic strains individually or in combination. Assuring that products contain these specific strains at the adequate amounts needed for the claim requires a novel approach outside of the tradition method of plate count enumeration. Plate count enumeration, while the industry gold standard for enumeration, is not strain specific and has a high method variability (upwards of 35%). This high variability is not able to detect small uniformity differences that may occur during production. This study shows that droplet digital PCR (ddPCR) is a novel technology that can be used to enumerate individual strains in a multi-strain product, even if those strains are closely genetically related. Three strains, two Bifidobacterium animalis subsp. lactis strains, Bl-04™ and HN019™, and one Lactobacillus rhamnosus, of an eleven strain dietary supplement blend were monitored over a large production run, with 16 samples taken throughout. Method variability and sample variability was measured throughout the production samples. Method variability contributed 9 to 13% variability, which was strain dependent. The overall uniformity of the samples ranged from 4% (Bl-04™) to 8% (HN019™) and showed no significant increase or decrease over time. This method was able to detect statistical differences (ANOVA, Minitab, Inc State College, PA) between individual samples, however due to the increase sensitivity of the method, the variability of the individual strains is acceptable according to industry standards. Overall, this novel method of using ddPCR to monitor the uniformity of a dietary supplement blend has proven to be strain specific and sensitive enough to evaluate a production run for minor blending issues.
Abstract Title: Mucosal Immune Recognition of Recombinant Lactobacillus Acidophilus and the Effect on the Microbiome Community Structure

Primary Author Block: B. Eklund, G. Dean, A. LaVoy, M. Smith, Z. Abdo; Colorado State Univ., Fort Collins, CO

Abstract Body:
Background: There is growing evidence that the intestinal microbiome influences many aspects of an individual’s health, including maintenance of intestinal homeostasis, nutrient processing, and maturation of the mucosal immune system [1]. This relationship is influenced by numerous factors such as diet, use of antibiotics, age of the host, and inflammation in the gut [1]. However, the interactions between the microbiome and the mucosal immune system have yet to be documented, especially throughout the course of vaccination [2]. We are exploring recombinant probiotic bacterium L. acidophilus as a platform for orally delivered vaccines against mucosally-transmitted pathogens, including HIV-1. Methods: We have inserted a linear epitope from the membrane proximal external region (MPER) of the HIV-1 envelope protein into the highly expressed bacterial surface layer protein (SlpA) of L. acidophilus. The fecal microbiome, fecal IgG, and serum IgA of BALB/c mice were observed throughout the course of 12 weeks, with six doses of different vaccine strains every two weeks. We then used Fluorescence-activated cell sorting and 16S rDNA sequencing to characterize microbes coated with secreted-IgA. Results and Conclusions: Our results demonstrate induction of both MPER-specific and SlpA-specific antibodies in serum and mucosa. We also saw significant shift in the microbiome community structure throughout the vaccination course. By differentiating between bacterial taxa coated with secreted-IgA, we can further investigate interactions between the microbiome and the mucosal immune system, and correlate community structure with the observed immune response to our recombinant vaccine.
Abstract Title: Evaluating the Colonization Potential and Cytotoxicity of -Bacillus Species On Human Colon Adenocarcinoma Caco2 Cell Lines

Primary Author Block: N. Mojgani, N. Vaseji; Razi Vaccine and Serum research Inst.-Agriculture Res., Ed. and Extension Organization, Karaj, Iran, Islamic Republic of, 2Animal Sci. Res. Inst. -Agriculture Res., Ed. and Extension Organization, Karaj, Iran, Islamic Republic of

Abstract Body:

Background: Adherence to mucus and intestinal epithelial cells is an essential criterion for selection of probiotic strains for human use. Methods: Bacillus megaterium (TA008 and TA009), Brevibacillus brevis (TA010) and Bacillus subtilis TA049 isolated from local honey samples (Razmgah et al 2016), were screened for their auto-aggregation, co-aggregation and cell surface hydrophobicity characters. The colonization potential of the isolates was assessed based on their adherence to human enterocyte-like Caco-2 cell lines and the number of adhered bacteria determined microscopically and colony count after trypsinization. Cytotoxicity percentage was determined by measuring survival and or death rate of Caco-2 cells after exposure to the mentioned Bacillus isolates. Each experiment was performed with three replicates. Data were analyzed with Statistical Analysis System Software (SAS) with glm procedure at significant level %0.05. Results: The auto-aggregation, co-aggregation and hydrophobicity percentage of the tested Bacillus species varied significantly, with B.megaterium TA008 and B.subtilis TA049 showing the highest autoaggregation and hydrophobicity percentages. B.megaterium TA009 and B.brevis TA010 demonstrated least auto-aggregative property and cell surface hydrophobicity. The co-aggregation properties of the species differed with the pathogenic strains and B.subtilis TA040 co-aggregated strongly with Salmonella typhi, whereas B.megaterium TA008 showed strong co-aggregation phenotype with Enterococcus faecalis. Auto-aggregation property of the isolates in study appeared directly related to adhesion ability and the isolates possessing higher auto aggregation percentage were also more adhesive to human enterocyte-like Caco 2 cell lines. All three isolates in study appeared non-cytotoxic to the intestinal epithelial cells and their cytotoxicity percentage was recorded below 15%, compared to the high cytotoxic nature of B.cereus ATCC 14579 (85.59%). Conclusions: B.megaterium TA008 and B.subtilis TA049 were able to inhibit, displace and compete significantly with the pathogens and thus might be suitable candidates for inhibition of tested pathogens. Further studies regarding the absence of virulence factors and in vivo safety analysis of the isolates are essential before we could propose their use as probiotic for future applications in food, feed or biotechnology industry.
Abstract Title:
Comparison of Plate Count Enumeration, Flow Cytometry and Droplet Digital Pcr on the Probiotic Strain Lactobacillus Acidophilus Ncfm®
Primary Author Block:
Abstract Body:
The World Health Organization (WHO) currently defines probiotics as “live micro-organisms which, when administered in adequate amounts, confer a health benefit to the host”. The question of how to accurately enumerate these micro-organisms has recently been raised because of the invention of novel technologies and increased understanding of how probiotic organisms provide health benefits. The current gold standard for enumeration in the probiotic industry is plate count enumeration. This method counts actively dividing cells through colony growth on special nutrient agar. Plate count enumeration has several drawbacks including long time to results (3-5 days), high variability (15-35% RSD) and the inability to differentiate species and strains. This study evaluates the novel application of flow cytometry (FCM) and strain-specific droplet digital PCR (ddPCR) for their effectiveness of enumerating probiotic culture concentrate and compares it to the traditional method. Ten different production lots of Lactobacillus acidophilus NCFM® were enumerated using plate count, flow cytometry and ddPCR and analyzed with Minitab (State College, PA: Minitab, Inc.). Results showed that each produced counts that were statistically different from each other, but were similar enough to warrant consideration as new enumeration methods because of their benefits. FCM counts intact cells that produce dye-adhering proteins, which can then be counted by the flow cytometer, is less variable 3% RSD and takes mere hours. ddPCR counts cells that are live and intact, due to the enumeration of genetic targets after DNA liberation. This method also takes hours vs. days, is the least variable (2% RSD) and most importantly, is able to differentiate strains. While it is challenging to make a direct comparison of these methods due to each targeting a different part of the cell physiology, however each of these methods hits upon a key part of the World Health Organization definition of a probiotic and may be viable options for alternative enumeration of probiotic bacterial cultures.
Abstract:
Microbiological Quality and Performance of Feeds Formulated with Graded Levels of Spent Brewers' Yeast (Saccharomyces cerevisiae) on Broiler Chickens
Primary Author Block:
Abstract Body:
Two hundred 14 day old “Sayed” broiler chickens were fed feeds formulated with spent brewers’ yeast (Saccharomyces cerevisiae) in graded levels (0, 5, 10, 15 and 20%) for 50 days. The aim of the study was to determine the potential of spent brewers’ yeast as alternative source of dietary protein replacing soya bean in poultry feeds. The objectives of the study were to assess the microbiological quality of the formulated feeds and using them to determine their effect on growth performance of broiler chickens. The experiments were in a completely randomized design with five treatments of 40 birds replicated into four of 10 birds each. Feed and water were provided ad-libitum. Mean total counts of formulated feeds ranged from 6.56 – 9.70 x 106 and 3.45 x 102 – 5.50 x 103 cfu/g for aerobic bacteria and fungi respectively. Eight genera of bacteria were isolated which included Bacillus, Staphylococcus, Escherichia, Proteus, Salmonella, Pseudomonas, Shigella and Klebshiella. Fungal genera included Aspergillus, Penicillium, Rhizopus, Mucor and Geotrichum. Final weight of birds ranged from 1962.50 – 2062.50g in the dietary groups. The economics of production showed lowest feed cost (N279.92) at 20% spent yeast replacement. Bacterial load did not affect performance negatively. Spent brewers’ yeast enhanced growth performance with reduction in feed cost and can be used to replace soya bean.
Abstract Title: Metagenomically Comparison of Bacterial Communities in Complete Gliadin-Degraded Sourdough (Khamir) Samples and Non-Degraded Samples

Primary Author Block: H. Sakandar, M. Imran; Quaid-i-Azam Univ., Islamabad, Pakistan

Abstract Body:

Background: Gluten intolerance is one of the food related disorders which is very common in occidentals but now it is also prevailing in orientals. However, it is still very uncommon in Asians especially Pakistan, due to their diet habits. Wheat is the staple food of Pakistan and it is mainly consumed in form of Khamiri Roti (autochthonously fermented sourdough bread). This study was conducted to investigate the comparison of bacterial communities in gliadin-degrading sourdough (Khamir) samples (SD2) and non-gluten degrading samples (SD1).

Methods: Fifty locally fermented sourdough samples were collected from various cities of Pakistan. Gliadin degraded samples were analyzed by FTIR analysis (Fig 1) and selected for metagenomic analysis by Illumina Miseq plate-form and xlatex was used to create relative abundance graphs and principle component analysis (PCA).

Results: It was observed that Proteobacteria (50.65%) and Actinobacteria (6.70%) phyla were in more abundance as compared to Firmicutes (42.53%) in SD2 while Firmicutes (83.44) were in more abundance in SD1 than Proteobacteria (14.97 %) (Fig 2). Effects were more prominent with lower taxonomic levels. 16S ribosomal RNA sequence also disclosed that Lactobacillus genera is the core genera in SD1 and SD2, 52.13 and 33.73%, respectively. However, second most abundant genera in SD1 and SD2 was Weisella (27.15%) and Psychrobacter (21.53%), respective (Fig 4). It was revealed that SD2 and SD1 samples have 15 and 9 different genera, respectively while 52 genera (Fig 3) in common were present in both. Shannon and Simpson indices (Fig 5) indicated that SD2 has more diversity compared to SD1. Different clustering of genera was observed in SD1 and SD2 by PCA graph (Fig 6).

Conclusion: Contrarily, sourdough samples (Khamir) had different bacterial communities as compared to previous studies of other authors. This study can be helpful to apply specific bacteria consortia to develop gliadin free food product.
Comparison of Raw Milk Microbiota in Cows with Staphylococcus aureus Positive and Negative Quarters

Primary Author Block:
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Abstract Body:
Background: Staphylococcus aureus (SA) intramammary infection is an important cause of bovine mastitis. Dysbiosis (microbial imbalance) has been associated to some bacterial diseases. The aim of this study was to determine if any specific raw milk microbiota signature could be associated to a natural SA infection. Because microbiota are notoriously variable from one individual to another, we paid particular attention to the microbiota of SA positive and negative quarters within the same cow. Methods: Lactating cows from a commercial farm in Canada were first screened for the presence of SA in each quarter. To be selected, a cow needed to have one quarter with more than 100 CFU of SA/mL of milk, and one SA negative quarter. i.e., no SA colony for three consecutive samplings conducted at weekly interval. Four cows were selected and the microbiota of 8 quarters were analyzed. Milk was collected from those quarters and frozen on the same day. DNA was extracted using the PowerFood kit from Qiagen. Duplicates of samples were then amplified by qPCR with primers selecting for the V1-V2 region of the 16S RNA gene and barcoded before sequencing by Illumina MiSeq. Sequences were treated with the QIIME2 pipeline. As we wanted to analyze the impact of SA relative abundance on the milk microbiota, and because milk bacteriology tests may vary from time to time, samples were strictly classified according to the percentage of SA sequences detected (vs total sample sequences). A SA prevalence of 0 to 2% was considered a SA negative quarter (SA-) while SA+ samples had a SA prevalence that varied from 5 to 91%. Results: The richness and the diversity of the samples were evaluated with the Chao1 and the Shannon indexes. When comparing the microbiota of all SA+ samples to SA- samples, Shannon and Chao1 indexes were significantly lower in SA+ samples (p = 0.04 and p = 0.01, respectively). Individually, one cow stood out with a significantly lower Shannon index (p = 0.002) for its SA+ quarter vs its SA- quarter. That cow showed by far the highest milk somatic cell count (5 million vs 116-204×103 cells/mL for the other cows). Interestingly for all analyzed quarters, we observed an inverse correlation between the prevalence of Corynebacterium sp., Aerococcaceae, Staphylococcus hominis and Leuconostocaceae and the prevalence of SA. Conclusion: The presence of SA within the milk of a quarter can have an influence on other bacteria composing the microbiota, therefore modifying its diversity and richness. It remains to be seen if some bacterial species can play a protective role against SA.
Session Title: AES11: Microbiology of Food, including Spoilage, Fermentation and Probiotics: Microbial Ecology, and Microbiomes of Foods
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science

Abstract Title: Metagenomics-Based Optimization of Campylobacter Isolation from Chicken Carcasses
Primary Author Block:
J. Kim1, H. Jung2, H. Park1, J. Kim1, S. Cho1, H. Shin1, S. Ryu1, B. Jeon1; 1Seoul Natl. Univ., Seoul, Korea, Republic of, 2Sejong Univ., Seoul, Korea, Republic of

Abstract Body:
Background: Campylobacter is the leading foodborne bacterial pathogen and the primary cause of Guillain-Barré syndrome. Because contaminated poultry meat is an important vehicle transmitting Campylobacter to humans, it is important to accurately detect and measure the levels of Campylobacter contamination to ensure food safety. However, the fastidiousness of Campylobacter often generates wide variations in the detection frequencies of Campylobacter from foods. In addition, competing microflora during selective enrichment steps further hampers Campylobacter isolation. To overcome the limitations, we optimized the procedures for Campylobacter isolation using Next-Generation Sequencing (NGS).

Methods: Twenty chicken samples from different geographical locations in Korea were divided into half and enriched with Bolton and Preston medium containing selective supplements. Total bacterial DNA was extracted from each enriched microflora, and the 16S rRNA was sequenced using the Illumina MiSeq platform. With 40 chicken samples, the frequencies of Campylobacter isolation of four medium combinations [Bolton broth-Bolton agar (BB-BA), Bolton broth-Preston agar (BB-PA), Preston broth-Bolton agar (PB-BA), and Preston broth-Preston agar (PB-PA)] were compared. Colonies on the selective agar were randomly chosen and subjected to 16S rRNA sequencing. Results: Bolton and Preston-selective enrichments generated diverse microflora, and only 32.4% of OTU types were shared between the media. The inter-individual difference of Bolton enrichment (Unweighted; P=0.004) were less than that of Preston enrichment. Enterobacteriaceae, particularly extended-spectrum beta-lactamase (ESBL) Escherichia coli, was highly prevalent in Bolton media that contains cefoperazone. Interestingly, the sequence of combining the selective media significantly affected the isolation frequencies; BB-PA (60.0%), PB-PA (27.5%), PB-BA (22.5%), and BB-BA (2.5%). Primarily due to less effective capabilities of Bolton in controlling ESBL E. coli, using Preston agars as the second selective media yielded better isolation frequencies than Bolton agars.

Conclusions: By using NGS, this study optimized the procedures of Campylobacter isolation only with Bolton and Preston media. To the best of our knowledge, this is the first metagenomics-based approach to optimize a protocol of bacteria isolation and provides novel insights about how NGS can be utilized in the optimization of protocols for the isolation/detection of pathogenic bacteria.
Abstract Title:
Revealing Flavor-Functional Core Microbiota in Chinese Light Aroma Type Liquor Fermentation

Primary Author Block:
Q. Wu, S. Wang, Y. Xu; Jiangnan Univ., Wuxi, China

Abstract Body:
Traditional fermented foods are very popular all over the world, because their unique flavor characteristics. It is generally accepted that the primary flavors of most fermented foods are produced by a few microbes, which are flavor-functional core microbiota. But the correlation between flavors and flavor-functional core microbiota is still poorly understood. Here, Chinese light aroma type liquor fermentation was taken as a model of a typical food process. Through high-throughput sequencing, 14 bacterial and 12 fungal genera defined to be the dominant microbiota with an average abundance over 1%. And 41 flavor compounds were detected during the liquor fermentation. In the dominant microbiota, seven genus (Lactobacillus, Pichia, Geotrichum, Saccharomyces, Rhizopus, Candida, and Clavispora) were strongly correlated (ρ > 0.6) with 30 flavor compounds. Thus, these seven genera were identified as flavor-functional core microbiota during the fermentation. In addition, variation partitioning analysis, partial redundancy analysis, and Mantel tests was proposed that moisture, temperature, ethanol, lactic acid, reducing sugar, acetic acid content and pH were major attributes affecting the core microbiota, with the explanatory variance of 87.86%. This method offers a powerful reference for finding flavor-functional core microbiota in food fermentation. Meanwhile, it would be beneficial for the establishment of a tractable and stable food fermentation. Keywords: Fermentation, metabolism, microbiota Acknowledgment: We gratefully acknowledge the National Natural Science Foundation of China (31530055, 31371822), the National Key R&D Program (2016YFD0400503), the Priority Academic Program Development of Jiangsu Higher Education Institutions, the 111 Project (No. 111-2-06).
Abstract Title:
Assessment of the Aerobic Microbiota in Fresh Cucumber and Commercially Fermented Cucumber Pickles Brined with 6% Sodium Chloride
Primary Author Block:
I. M. Perez-Diaz1, J. Hayes1, E. Medina2, A. Webber1, N. Butz3, A. Dickey4, J. Lu5, M. Azcarate-Peril3; 1USDA-Agriculture Res. Service, Raleigh, NC, 2NC State Univ., Raleigh, NC, 3Univ. of North Carolina-Chapel Hill, Chapel Hill, NC, 4North Carolina State Univ., Raleigh, NC, 5Kennesaw State Univ., Kennesaw, GA
Abstract Body:
The limited documentation of the cucumber fermentation microbiome has impeded the understanding of the role of microbes on the quality of finished products. We aimed at characterizing the microbiome of 19 fresh cucumber samples representing 4 commercial types and 2 commercial fermentation cover brines, using culture dependent and independent techniques, with emphasis on aerobic Gram-negative bacteria. Dissolved oxygen was monitored during fermentations. Selective plating was used to screen for relevant microbes in 9 fermentation cover brine samples. Insubstantial microbiome variations were observed among fresh cucumber types with Rhizobium (31.04%), Pseudomonas (14.08%), Pantoea (9.25%), and Stenotrophomonas (6.83%) dominating. Lactic acid bacteria (LAB) were found to less than 0.37% relative abundance. Maximum oxygen intake in cucumber fermentations was observed on days 1-3 of fermentations and followed by undetectable levels of lactose fermenting bacteria. Fermentation cover brine samples collected on day 1 harbored the same bacteria found in fresh cucumbers, except for Rhizobium, a strict aerobic bacterium. Cucumber fermentation cover brines also contained Comamonas, Acinetobacter, Wautersiella, Microbacterium, Flavobacterium, Enterobacter, Ochrobactrum, Citrobacter and Kluyvera in relatively high abundance. Incidence of LAB remained below 4.0 % on day 3 of the fermentations brined with 6% NaCl. Colony counts for presumptive Klebsiella and Pseudomonas from fermentation cover brine samples reached 2.58 ± 0.33 and 2.46 ± 0.67 log of CFU/mL, respectively, in one third and more than half of the 9 tanks scrutinized with selective media. Both genera were found in cover brine samples with pH values at 4.08 ± 0.14 and lactic and acetic acids concentrations of 27.84 ± 6.88 and 3.31 ± 0.63 mM, respectively. We aim at elucidating if the low incidence of aerobic bacteria in commercial cucumber fermentations, in particular Pseudomonas and Enterobacteriaceae, impact the quality of fermented cucumbers.
The Fine Level Diversity, Dynamics and Function of the Cocoa Bean Fermentation Microbiome: A Sys. to Study and Model Microbial Communities

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Background: Cocoa bean fermentation is a crucial post-harvest process for the generation of good quality chocolate. This process is catalyzed by environmental microbes that colonize the cocoa pulp in a predictable microbial succession. Here we present a robust study of the population dynamics of the microbial cocoa fermentation using high-throughput sequencing methods that allow us to evaluate the inter- and intra-specific diversity of the microbial community, the dominance and transitivity of strains (within and between regions), their interactions. Methods: Three cocoa farms were selected for the study, each representing an important cacao producing region in Colombia. Sampling was conducted during two different periods in 2016. During each period cocoa beans were collected during the whole fermentation process, with a 12 hour from the upper and intermediate zones of wooden fermenters. A total 126 cocoa bean samples were collected. DNA extraction and library preparation was done for 16S rRNA genes and ITS region. All libraries were sequenced with the V2 kit using a MiSeq platform (Illumina). Results: Sequencing of 16s rRNA and ITS libraries generate a total of 3’560.120 paired reads. The taxonomic classification of reads for all samples showed a reduced diversity at the 97% OTUs level. The microbial community composition at the 97% OTU-level was similar in all fermentation systems (department independent) and showed the expected microbial succession, were microbial abundance is dominated by enteric bacteria then transition to Lactic Acid Bacteria (LAB) and finally to Acid Acetic Bacteria(AAB). The analysis of oligotyping divided each OTU, into finer groups (oligotypes groups), for Yeast, Enterobacteria, LAB and AAB groups, 12, 6, 11 and 19 oligotypes were found respectively. Interestingly the dominant strains seem to be the same on all evaluated farms. While transient strains appeared only at the transition of the microbial succession suggesting more abundant and diverse resources. Conclusions: In conclusion, these results show that there is a larger that previously reported microbial diversity in the Cocoa fermentation process and highlights the importance of fine level diversity tools to monitor the dominance and resilience of microbial populations. Such approaches are necessary for the improvement of fermentation protocols and the development and validation of starter cultures.
Abstract Title:
Phenotypic Character of Culture Dependent Molecular Grouping of Bacillus Species from Ready to Eat Fermented Parkia Biglobosa Condiments from Nigeria

Primary Author Block:

Abstract Body:
This study aims at identifying the phylogenetic relationship and strains sub-typing of Bacillus species isolated from ReadyToEat naturally fermented Parkia biglobosa condiments from different GeoPolitical Zones of Nigeria. Polyphasic genomic approaches were used for study. Cultural and biochemical methods of characterization were employed with the molecular technique limited to the use of 16S rRNA primer and OPR 13-Randomly Amplified Polymorphic DNA (RAPD-PCR). A total of thirty-eight (38) isolates of Bacillus species were obtained from 48 commercial samples of RTE condiments including Bacillus subtilis, Bacillus pumilus, Bacillus licheniformis and Bacillus cereus with percentage frequency of occurrence of 100.00%, 58.33%, 33.33% and 25.00% respectively. DNA sequencing of the highly variable V3 region of the 16S rRNA genes obtained from PCR-DGGE identified species related to Bacillus subtilis as consistent bacterial species in the RTE samples. The result of 16S rRNA shows that the RTE are composed of clonally related Bacillus species which have similar clusters and gene sequencing identified the strains as Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus cereus, Bacillus licheniformis, Bacillus pumilus, and Brevibacillus formosus. The phylogenetic analysis conducted showed five distinct clusters with genetic relatedness among B. subtilis and B. amyloliquefaciens strains. Randomly amplified polymorphic DNA (OPR 13-RAPD-PCR) further discriminated between the Bacillus species and confirmed B. subtilis and B. amyloliquefaciens as the dominant Bacillus species associated with RTE of Parkia biglobosa naturally fermented condiments and revealed high strains genetic relatedness. This information is essential for selection of starter cultures with desirable functional attributes to guarantee product consistency and safety quality of traditional fermented foods. It also suggested the need for development of controlled fermentation processes and good manufacturing practices (GMP) for Parkia biglobosa condiments production to improved shelf life.
Session Number: 256
Session Type: Poster
Session Number: 256
Session Type: Poster
Session Title: AES15 - Synthetic Biology
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 6902
Poster Board Number: SATURDAY - 906

Abstract Title:
Micro-Emulsion Droplets As A Novel Platform to Study Evolution of A Synthetic E. coli Predator-Prey Population
Primary Author Block:
R. Ganiga Prabhakar, R. Alnahhas, M. R. Bennett, Y. Shamoo; Rice Univ., Houston, TX
Abstract Body:
Competition for resources is one of the most fundamental driving forces of natural selection and has markedly influenced the evolution of microbial communities. As a consequence of competition, bacteria have accumulated the ability to produce various bioactive secondary metabolites under specific environmental conditions. Some of these pathways are activated only under certain stress conditions or remain largely silent. Activation of these cryptic pathways can provide us with insights into how microbial communities interact as well as access to potentially novel bioactive metabolites for industrial and biomedical use. Encapsulation of bacteria in emulsion droplets allows us to study single cells and all the metabolites secreted by those cells. We have used approaches from synthetic biology to engineer a predator-prey system in E. coli to study how environmental stress can be tied to the activation of a cryptic pathway in a predator to gain resources from another strain of E. coli that serves as the prey. As a proof of principle, predator and prey are co-cultured under nutrient limitation conditions within emulsion droplets. A predator evolved to activate the engineered cryptic pathway produces quorum sensing (QS) molecules that communicates with the prey cells present within the droplet. Prey is engineered to activate suicide lysis genes in response to the detection of QS molecules present in the media. We have introduced a single nucleotide polymorphism (SNP) within the predator to knockout production of the essential QS molecule required for killing prey. A predator population that reverses the SNP can grow to a higher density compared to unevolved predator within the droplets by killing the prey cells. Spatial segregation imparted by the emulsion droplet prevents the diffusion of common resources between droplets carrying evolved and unevolved predators. By iterating the growth of evolved predator in emulsion, we can increase its population density to detection level. If successful, this system of competition based directed evolution can be extended for harnessing the evolutionary power of bacteria to produce molecules of interest under defined synthetic stress conditions.
Abstract Title:
Dual-Targeted Cas9 Endonuclease DsDNA Cleavages Facilitates Allelic Exchange for In Vivo Engineering of Virulent Sau Bacteriophage
Primary Author Block:
D. J. Ferullo, J. Warner, J. A. Radding; EnBiotix, Inc, Cambridge, MA
Abstract Body:
Background: An increase in antibiotic resistant bacterial infections has renewed interest in phage therapy as an alternative to traditional antibiotic treatments. We are exploring the development of engineered bacteriophage to treat biofilm-associated prosthetic joint infection (PJI) caused by Staphylococcal aureus (Sau). We have developed an in vivo CRISPR/Cas9-based engineering system that produces and enriches for recombinant bacteriophages in Sau. The system was validated by successful construction of a bacteriophage containing the major capsid protein (MCP) gene of a second closely related phage. Currently, we are employing this strategy to construct phages that contain genes encoding biofilm-dispersing enzymes and/or optimized receptor-binding proteins. Methods: Repair template, carrying the foreign MCP gene, and Cas9-encoding plasmids were constructed as previously reported[1, 2]. An engineering Sau strain, RN4220 harboring the aforementioned plasmids, was subjected to infection by bacteriophage GRCS in liquid culture at an MOI of 10 for 4 hours. Recovery of phages was achieved by double agar overlay assays. PCR, restriction digestion, and Sanger sequencing signatures were used to identify recombinant phages. Bioinformatics were performed using Geneious 11.0.2. Results: Immunity to GRCS infection is rendered when directing Cas9 to cut both 5’ and 3’ regions of the MCP gene of GRCS (MCPGRCS) without incidence of CRISPR Escape Mutants (CEMs). Provision of a repair template plasmid comprising GRCS homology and a heterologous MCP gene from phage 44AHJD (MCP44AHJD) allowed for the production of bacteriophage with MCP44AHJD in place of MCPGRCS. Recombinant phage recovery was 100% when supernatants were plated onto Sau carrying the Cas9 plasmid. Conclusions: Introduction of two distal cut sites to facilitate homologous recombination-based phage editing is optimal for the generation and recovery of engineered phage. This method can be used to produce engineered bacteriophage containing indels and other mutations. Accordingly, production and enrichment of bacteriophages containing genes encoding antimicrobial payloads, altered structural profiles, or with altered gene regulation for optimizing phage utility as therapeutics is achievable.
Abstract Title:
Bioconversion of Acrylonitrile to Acrylamide Using Non-Immobilized and Immobilized Cells of 
Rhodococcus Rhodochrous Dap 96253 

Primary Author Block:
B. Galbreath, K. Cannon, M. de la Croix, N. Amadason, G. Pierce; Georgia State Univ., Atlanta, GA

Abstract Body:
Background: Acrylamide (AMD) is an important commodity chemical that is used in coagulators, water treatment, soil conditioners, mineral refining, paper treatment, adhesives, paints, petroleum recovering agents, and in certain laboratory procedures. It is often shipped as an aqueous solution (30-50% w/w acrylamide). To bypass the cost which occurs with shipping a solution that is 70-50% w/w water, one can locally produce AMD or poly-AMD using microorganisms to convert acrylonitrile (AN). Rhodococcus rhodochrous DAP 96253 uses a nitrile degradation pathway involving the enzyme nitrile hydratase. Nitrile hydratase catalyzes hydrolysis of the nitrile to an amide following the equation: RC≡N + H2O \rightarrow RCONH2. Free whole cells and immobilized cells, using either glutaraldehyde-polyethylenimine (GA-PEI), calcium-alginate, or polyacrylamide (PAM), of R. rhodochrous DAP 96253 were used to compare the production of AMD from AN and to obtain solutions of 40% w/w and 20% w/w, respectively, to compete with commercially-produced solutions. Methods: Induced cells of R. rhodochrous DAP 96253 were placed into a small-scale bioreactor under constant mixing. Acrylonitrile was added into the bioreactor at varying rates. Samples were taken every 30 minutes to show the conversion of AN to AMD over time. These samples were analyzed using gas chromatography-flame ionization detection. Results: Production of bio-AMD varied from 0.63-49.92% (w/w) based on whether the cells were induced, non-induced, grown on plates, harvested via fermentation, whole cells, or immobilized cells and the rate at which AN was added. All runs took place at room temperature. Conclusions: Acrylamide can be produced by converting AN in the presence of R. rhodochrous DAP 96253 by using its enzyme, nitrile hydratase, as a catalyst for the reaction. Consistent production thus far (>45% w/w) has been achieved with whole cells induced with cobalt and urea produced via fermentation and a 6-hr run time. By using this method of bioconversion, companies save money on buying/shipping AMD commercially and produce higher quantities than what is commonly available commercially, 40% w/w.
Session Number: 256
Session Type: Poster
Session Number: 256
Session Type: Poster
Session Title: AES15 - Synthetic Biology
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 4262
Poster Board Number: SATURDAY - 909

Abstract Title:
Queueing Coordination of Type II Toxin-Antitoxin Systems
Primary Author Block:
H. S. Deter, N. C. Butzin; South Dakota State Univ., Brookings, SD
Abstract Body:
Bacterial persistence can be a major contributing factor when antibiotic treatments fail, particularly in cases of chronic and reoccurring infections. Persistence is a microbial phenotype characterized by metabolic dormancy and multidrug tolerance that functions as a survival strategy in stressful and harsh environments. The persister subpopulation survives sudden events that kill the vegetative population because persisters have slower translation and other metabolic processes compared to vegetative cells. Proteases are important for the development of persisters, and the Lon protease’s role is best described. In the absence of Lon, persistence levels decrease to near zero. Proteins processed by Lon include those in toxin-antitoxin (TA) systems, which are also related to persistence. In type II TA systems, an unstable antitoxin protein binds to and disables a cognate toxin protein, but when toxins are at a higher level than their cognate antitoxins, free toxin activity slows metabolism and triggers persistence. Studies have shown that multiple TA systems are coordinated at the transcriptional level, and the same proteases often degrade their proteins. Proteases play an important role in the proteolytic coordination of cellular processes, which results from biological queueing (waiting-lines; bottlenecks) caused by limited processing resources in a cell. We hypothesize that persister levels could be coordinated through the formation of protease bottlenecks. We take a synthetic biology approach to test this hypothesis by producing seemingly unrelated, fluorescent proteins containing different amino acid tags targeted to specific proteases. The overproduction of these tagged proteins creates a proteolytic queue that slows the degradation of many proteins, including antitoxins. The resulting differences in protein concentration, in turn, affect persistence levels in the population.
Continuous Real-Time Detection of the Camp Response Element (Cre)-Mediated Gene Activation in Saccharomyces cerevisiae Using An Autobioluminescent Reporter

Primary Author Block:
A. Young1, F. Ji1, T. Xu1, D. Close2, S. Ripp1; 1The Univ. of Tennessee, Knoxville, TN, 2490 BioTech, Knoxville, TN

Abstract Body:
The cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) signaling pathway plays a critical role in the regulation of cell cycle, metabolism, and stress response in Saccharomyces cerevisiae. Regulation of the cAMP-PKA pathway modulates the activity of cAMP response element binding protein (CREB), which binds to the CRE cis-regulatory sequence located within target genes. The ability to continuously monitor the activity of CRE-mediated gene expression in real-time in live S. cerevisiae cells will provide a facile means of investigating the dynamics of critical cellular processes. This study developed an autonomously autobioluminescent bioreporter for monitoring CRE activation and demonstrated its utility to monitor cAMP-PKA induction in response to glucose starvation. This recombinant yeast bioreporter was based on a synthetic bacterial luciferase reporter gene cassette (lux) that can produce the luciferase as well as the enzymes capable of self-synthesizing the requisite substrates for bioluminescent production from endogenous cellular metabolites. As a result, bioluminescent signal production is generated continuously and autonomously without cell lysis or exogenous reagent addition. By linking the expression of the autobioluminescent lux reporter to cAMP-PKA pathway activation via the use of multimeric CREs, the resulting bioreporter emitted bioluminescent signal in a glucose concentration-dependent manner. Exposure to glucose-limited conditions at 0.02% and 0.2% produced significantly higher bioluminescence relative to the glucose-sufficient controls (2%) within 5.5 and 7.5 hours of growth, respectively. After 12 hours of growth, bioluminescent expression was induced by approximately 75-fold in cells grown in 0.02% glucose, while 0.2% glucose only induced light production by 45-fold in 12 hours. The reporter cells also responded to changes in glucose concentration, indicated by the real-time detection of a rapid decrease in bioluminescence upon recovering glucose-starved cells in glucose-sufficient medium. Within 1 hour of recovery in 2% glucose, previously starved (0.2% glucose) cells produced approximately 85% less bioluminescence compared to unrecovered control cells. These results demonstrate that using the lux reporter cassette it is possible to continuously monitoring the dynamics of the CRE-mediated gene expression in response to varying stress conditions and provides a potential high-throughput platform for screening of CRE inducers and inhibitors.
Abstract Title:
Metabolic Engineering of Pseudomonas Sp. Lfm046 Producing Biopolymer / Polyhydroxyalkanoates from Glucose

Primary Author Block:
J. Cardinali-Rezende, C. N. Kim, L. F. Silva, M. K. Taciro, A. Steinbüchel, J. C. Gomez; Univ. of São Paulo, São Paulo, Brazil

Abstract Body:
The Pseudomonas sp. LFM046 has been studied as biological platform for the production of biodegradable polyhydroxyalkanoate with medium-chain-length 3-hydroxyalkanoates (PHAMCL). The genome of LFM046 was obtained (Cardinali-Rezende et al. 2015) and the annotation of their genes was manually refined. The main pathways related to the glucose degradation and polymer biosynthesis are: Pentose Phosphate pathway (PPP), Embden-Meyerhof Parnas pathway (EMP), Entner-Doudoroff pathway (EDP) and Krebs Cycle. Since pfk gene (encodes phosphofructokinase), essential to EMP pathway, and gnd (6-phosphogluconate-dehydrogenase) gene, involved on PPP entrance, are absent in this strain, the EDP seems to be the main route for glucose catabolism. In addition to this, the metabolic flux analysis suggested that the efficiency in converting carbohydrate into PHAMCL by this strain corresponded to 60-70% of the maximum theoretical yield, probably due to a high flow rate in the PPP. Labelling pattern analysis of PHA monomer produced using 12C-Glucose (80%) and 13C-Glucose (20%) were compatible with the high flow in the PPP. An elementary mode analysis indicated that an increase in flow through the EDP may improve the carbohydrates conversion into PHA. Due to this, the objective of this proposal is the overexpression of edd and eda genes (EDP) and evaluates the impact of heterologous expression of gnd gene from Escherichia coli MG1655 (gndEc) and P. putida KT2440 (gndPp) in PHAMCL yield from glucose by Pseudomonas sp. LFM046. For this, genomic DNA of LFM046, MG1655 and KT2440 strains were extracted to amplification of edd (encodes 6-Phosphogluconate dehydratase) and eda (encodes 2-dehydro-3-deoxyphosphogluconate aldolase) genes from LFM046 and gnd genes from MG1655 and KT2440 strains. The genes were amplified by PCR, cloned in pBBRI-MCS2 plasmid and transferred into Pseudomonas sp. LFM046 by electrottransformations. Pseudomonas sp. strains LFM046::pBBRI-MCS2 (control), LFM046::pBBRI-MCS2-gndEc and gndPp were cultured in 200 mL of MSM containing 15 g l-1 of glucose, at 30°C and 150 rpm for 72 h. Recombinants strains presented the same yield of PHAMCL than control strain. PHAMCL composition was 3 mol % of 3HHx; 29 mol % of 3HO; 65 mol % of 3HD and 3 mol % of 3HDd. Recombinants strains LFM046-pBBRI-MCS2::eda and LFM046-pBBRI-MCS2::edd-eda also were obtained and will be cultured for analysis of PHAMCL production. These analyses will help us to understand the importance of these genes for the success of this Pseudomonas sp. in the PHA production to improve this process.
Session Number: 256
Session Type: Poster
Session Title: AES15 - Synthetic Biology
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 1990
Poster Board Number: SATURDAY - 912

Abstract Title:
Construction of Escherichia coli Proof-Of-Principle Strains Using Genome-Scale Metabolic Model As Platform

Primary Author Block:
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Abstract Body:
The conventional experimental trial and error approach in strain design for metabolic engineering has been established to be labor-intensive, expensive and/or time consuming strategy. However, advances in genome-scale metabolic modeling has open up novel platforms for construction of “proof of principle strains” that could serve as basis for discovery and design of robust microbial cell factories for targeted biosynthetic goals within shortest possible time. In this presentation, it will be demonstrated how we can use Escherichia coli genome-scale model as platform for the construction of proof of principle strains that enhances succinic acid production from glucose, and glycerol carbon sources. Examples will be on the identification of four (4) novel gene deletion targets for increasing succinic acid production in E. coli.
Abstract Title:
Expressing Bacterial Luminescence Genes in Human Cells to Develop Ready-To-Use Drug Screening Tools Suitable for Microgravitational Use Onboard the Intl. Space Station
Primary Author Block:
T. Xu1, S. Ripp1, G. Sayler2, D. Close2; 1The Univ. of Tennessee, Knoxville, Knoxville, TN, 2490 BioTech, Knoxville, TN
Abstract Body:
Human cell culture in microgravity promotes the natural formation of three-dimensional structures without necessitating exposure to exogenous scaffold materials. This endows cells with a more natural physiology and enhances drug discovery by enabling enhanced prognostication of compound efficacy. Unfortunately, the logistical costs of delivering the materials needed for firefly luciferase-based compound evaluation in low Earth orbit have obviated the use of microgravitational research platforms, such as the International Space Station, for this purpose. We are investigating the adaption of the bacterial luciferase gene cassette to serve as a reporter for human cell metabolic impact testing to overcome the logistical hurdles of space-based drug discovery. The bacterial luciferase gene cassette from Photorhabdus luminescens was modified for expression in human cells and co-expressed with a supporting oxidoreductase to enable continuous bioluminescent output concordant with the host’s metabolic activity level. A variety of cryogenic preservation and reanimation approaches were evaluated to develop a method whereby cells could be pre-packaged on Earth, stored at -80 °C, then thawed and injected into pre-prepared, drug compound-containing multi-well plates in-flight and used to continuously monitor metabolic activity. This method was tested against five therapeutic compounds with known metabolic impacts. Using the method developed in this work, compound-induced metabolic impacts can be tracked continuously for up to 24 hours. The observed timing of compound metabolic impacts remained consistent (p ≤ 0.08) and ANOVA of signal strengths from replicate assays ranged between 0.49 and 0.77 for all tested chemicals, demonstrating a high level of consistency. Our findings indicate that the use of continuous bioluminescence as a reporter tool produces the necessary detection characteristics to enable metabolic activity/toxicity testing in low Earth orbit. The self-contained nature of the system reduces the number of samples and reagents required and lowers testing costs by limiting the weight and space burdens imposed during delivery to the International Space Station. This system has been cleared for in-flight testing and is scheduled for delivery to the space station for validation on the SpaceX-14 flight in April 2018.
A Comprehensive Yeast Tool Kit with Novel Synthetic Regulation Sys. for Gene Circuit Construction and Delivery

Primary Author Block:
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Abstract Body:
Sophisticated behavior within cells manifests from multiple regulatory networks in which transcriptional factors (TFs) regulate gene expression while binding to their cognate operator sequences. For prokaryotic models, such as Escherichia coli, a number of TFs have been utilized to engineer synthetic transcriptional networks encoding toggle switches, oscillators, or logic operations. However, the creation of synthetic circuits has proven more difficult in eukaryotic systems due to the lack of suitable “parts” to use within genetic networks (i.e., promoters, activators, and repressors), as well as the lack of a standardized platform for DNA assembly and the flexible delivery of gene circuits. Here, we demonstrate a framework for building gene circuits and present a set of well-characterized DNA parts for use in Saccharomyces cerevisiae. For the assembly of novel gene circuits, we used Gateway® (Thermo Fisher Scientific) recombination and Gibson Assembly® (Synthetic Genomics) methods. Hierarchical assembly of gene circuits comprising multiple transcriptional units was mediated through unique 45 bp sequences. Characterization of various promoters and the rules of network design were evaluated by analyzing fluorescent protein expression (GFP and YFP) via flow cytometry. We also characterized a diverse set of promoters consisting of constitutive promoters, native inducible promoters, synthetic inducible promoters, synthetic promoters regulated by activators, and synthetic promoters regulated by repressors. Promoter expression was measured in MEFL units with the following results: 1) constitutive promoters demonstrated a wide range of strengths (up to a 100-fold difference); 2) the new inducible systems enabled an 11-fold change in expression; and 3) the activators/repressors showed a maximum 35-fold and 45-fold change of expression, respectively. In conclusion, this study demonstrates the feasibility of quickly and easily constructing gene circuits for delivery into S. cerevisiae, as well as the utility of a fully characterized set of diverse promoters, activators, and repressors. In addition, the study suggests that this system can be applied in constructing large-scale gene circuit libraries with reliable gene expression and designing logic operations for a complex network in S. cerevisiae.
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Session Primary Track: Applied and Environmental Science
Abstract Control Number: 4796
Poster Board Number: SATURDAY - 915

Abstract Title:
A Biol. Operational Amplifier (Bio-Opamp) in A Synthetic Microbiol Consortium
Primary Author Block:
J. Zeng1, J. Teo2, A. Banerjee1, J. Kim3, R. Sarpeshkar1; 1Dartmouth Coll., Hanover, NH, 2Massachusetts Inst. of Technology, Cambridge, MA, 3Korea Inst. of Sci. & Technology (KIST), Seoul, Korea, Republic of

Abstract Body:
Background: Several electronic circuit motifs and devices including oscillators, latches, logic gates, logarithmically linear circuits, and load drivers have been designed and ported to biological systems and applications. However, the operational amplifier, which is a fundamental and the most popular electronic device in negative-feedback and regulatory loops, has never been ported to biology.
Methods: By mimicking a typical three-stage-OpAmp, we designed a three-gain-stage biological operational amplifier (Bio-OpAmp). The first gain stage is a fast differential amplification stage with arabinose (non-inverting signal) and AHL (inverting signal) inputs generating two corresponding sgRNA outputs. The second gain stage is a slow differential stage that uses CRISPR-sgRNA complexes to produce AiiA and LuxI enzyme outputs. The third gain stage is a fast stage that uses AiiA and LuxI enzymes to degrade or produce AHL, respectively. The AiiA and LuxI enzymes also perform differential-to-single-input conversion to regenerate the AHL input which is fed back to the inverting terminal.
Results: The output AHL faithfully tracks the input arabinose, ignoring the external added AHL interference, over more than an order of magnitude of input dynamic range as would be expected in a high-performance control system with negative feedback. The three-gain-stage amplification, however, is a prime candidate for oscillation as for example in repressor cascades with negative feedback. We solved this problem by intentionally designed a dominant integrator time constant as in well-known operational-amplifier, such that protein production and degradation are relatively slow, but RNA and chemical inducer (arabinose and AHL) production and degradation are fast. Last but not least, the performance is achieved in a synthetic microbial consortium, which alleviates synthetic-circuit metabolic burden in each cell population. Conclusions: In this Bio-OpAmp, arabinose effectively serves as a control or set-point non-inverting input that determines the intended equilibrium and homeostatically-controlled value of the output AHL. Homeostasis is a universal phenomenon wherein a biological system maintains steady states of output variables rejecting disturbances from external conditions. Hence, our synthetic circuit with the AHL and arabinose halves has similar analogs in nature, which will be useful in molecular homeostasis in biotechnology and medicine.
Abstract Title:
Development of Stable Broad Host-Range Shuttle Vectors for Lactobacillus Engineering

Primary Author Block:
J. Spangler1, S. Walper2; 1Natl. Res. Council, Washington, DC, 2Naval Res. Lab, Washington, DC

Abstract Body:
Background: Continued innovation of molecular biology tools has made microbial engineering as a potential solution to critical problems a realistic possibility. Lab strains such as E. coli K12, however, can limit problem-solving potential due to its growth and survival characteristics as well as its utility outside of the laboratory. Engineering Lactobacillus species for eventual human use, on the other hand, would be more advantageous as they are generally regarded as safe (GRAS) commensals. Unfortunately many of these species are poorly characterized and lack adequate genetic tools, thus creating a barrier for consideration as engineering chassis. Using rational plasmid design and published proteomic data, we set out to develop a broad host-range shuttle vector to function in Lactobacillus species to demonstrate the feasibility of engineering these less common commensals. Methods: Shuttle vectors were created using In Vitro Recombination in E. coli to assemble different replicons, selective markers and promoters that would function in multiple genera and species. Reporter constructs were made similarly by identifying native constitutive promoters to express superfold GFP (sfGFP) using published proteomic data as a guide. Promoter strength was determined by fluorescence and RT-qPCR measurements over time, and survival studies were carried out to assess plasmid maintenance with the lack of selective pressure from antibiotics. Results: The most stable vectors across all tested species contained the pWV01 replicon or a truncated version. Dual antibiotic selection markers were crucial to the effortlessness transition between species. Five promoters were identified with varying expression levels in L. plantarum, the highest of which was confirmed in other tested species. In some species plasmids were maintained for up to 11 days without antibiotics.<u></u> Conclusions: Here we demonstrate a functional shuttle vector in both Gram-positive and -negative bacteria that can be used to engineer Lactobacillus species, among others. The assembly method and dual antibiotic selection markers together facilitate a user-friendly and cost-effective protocol with a short turnaround time from editing to testing in the target species, making the engineering of less common strains more feasible. The demonstrated plasmid stability is also a promising characteristic that could be exploited to observe long-term results without relying on genome editing techniques.
Simultaneous Degradation of Commingled Contaminants by A Microbially-Driven Fenton Reaction Operated in Fed-Batch and Flow-Through Reactor Modes

Primary Author Block:
Y. Toporek; Georgia Inst. of Technology, Atlanta, GA

Abstract Body:
Background: Organic solvents such as trichloroethylene (TCE) and perchloroethylene (PCE) are detected in contaminated soil and ground water near industrial sites that are often co-contaminated with the solvent stabilizer 1,4-dioxane and perfluorinated compounds (PFCs). Recent concern over commingled co-contaminants is driven by several factors, including toxicity to liver, kidney and central nervous system function, and recalcitrance to conventional degradation processes. Current remediation technologies such as photolysis, sonolysis, and enzymatic reductive dehalogenation are not cost-effective and can be limited by nutrient requirements and production of toxic intermediates. Chemical oxidation processes are attractive alternative remediation technologies due to high reactivity and relatively low cost. The objective of this study was to simultaneously degrade the commingled contaminants TCE, PCE, 1,4-dioxane, and perfluorooctanoic acid (PFOA) by a microbially-driven Fenton reaction in fed-batch and flow through reactor (FTR) configurations that operate at circumneutral pH and do not require continual addition of the Fenton reagents Fe(II) and peroxide. Approach: FTR advective flow columns were loaded with Fe(III)-reducing facultatively anaerobic bacteria, Fe(III)-coated quartz sand and fed artificial groundwater amended with commingled 1,4-dioxane, TCE and PCE. Fenton reaction optimization studies were performed on three contaminant-free columns: biotic with partially oxygenated media (A), biotic with degassed media (B), and abiotic with degassed media (C). Mathematical models were developed to predict co-contaminant degradation and validate experimental results under both fed-batch and flow-through reactor configurations. Batch and FTR testing under the same conditions is underway with PFOA-amended media. Results: The generation of reactive hydroxyl radicals via the microbially-driven Fenton reaction effectively degraded 1,4-dioxane, TCE and PCE in single, double and triple combinations in fed-batch and FTR reactor configurations. The contaminants were not degraded in control experiments lacking bacterial cells or Fe(III). Pumping oxygenated media through the FTR separated the FTRs into two zones: an aerobic zone in the first section of the column followed by an anaerobic zone in the second section of the column (most likely caused by microbial oxygen consumption). Contaminant degradation via the microbially-driven Fenton reaction was detected at the interface of the oxic and anoxic zones.
Abstract Title: Microbially-Catalysed Anaerobic Metal Redox Cycling by Acidophilic, Geobacter Sp. Feam09

Primary Author Block: O. M. Healy1, S. Antony-Babu1, G. Hollis1, J. Souchek1, B. LaMere1, R. V. Kiat1, D. Pan1, W. H. Yang2, W. L. Silver3, K. A. Weber1; 1Univ. of Nebraska-Lincoln, Lincoln, NE, 2Univ. of Illinois at Urbana-Champaign, Urbana, IL, 3Univ. of California-Berkeley, Berkeley, CA

Abstract Body: Nitrogen (N) mediated metal redox cycling has been identified in both aqueous and sedimentary/soil environments and occurs through coupled biologically and/or abiotically driven redox reactions. Together these reactions influence both N and metal biogeochemistry. An acidophilic (optimal growth pH 5.0), autotrophic Geobacter sp. strain FeAm09, was isolated from a series of Feamox enrichments initiated with Fe rich tropical forest soils (Luquillo Experimental Forest, Puerto Rico) under Fe(III) reducing conditions. Strain FeAm09 is capable of growth using soluble Fe(III), Fe(III)-NTA, as well as insoluble Fe(III) and Mn(IV) oxides as terminal electron acceptors with H2 as the electron donor and is also capable the reduction of nitrate (NO3-) to ammonium (dissimilatory NO3-reduction to ammonium or DNRA) coupled to the oxidation of Fe(II) or Mn(II). In addition to ammonium (NH4+), dinitrogen (N2) gas and nitrous oxide (N2O) were also formed in equal molar amounts to NH4+ with both Fe(II) and Mn(II) serving as the electron donor. The completed genome sequence of FeAm09 supports the reduction of NO3- to nitrite (napB) and subsequent nitrite (NO2-) reduction to NH4+ (nrfB). Genes responsible for N2 production have not been identified and strain FeAm09 grown on H2 with NO3- as an electron acceptor resulted in only NH4+ production. Together these results suggest that NO2- may be produced as an intermediate and abiotically reduced by Fe(II) and Mn(II) yielding N2O and N2, as supported by a series of experiments in which NO2- was added to treatments containing reduced Fe and Mn oxides. Only the live treatment reduced NO2- to NH4+, which indicates that NH4+ production coupled to metal oxidation is biological, while an abiotic reaction results in N2O and N2 production. Based on our results approximately half of the electrons are directed to NO3- respiration (DNRA). Genes within the reverse TCA cycle were identified supporting an autotrophic lifestyle (citB, frdB, fumB, icdI, korA). CO2 fixation was verified during metal oxidation through assimilation of 14C-labelled CO2 into biomass under metal reducing and metal oxidizing conditions. The ability of FeAm09 to reduce NO3- to NH4+ as the sole NO3-reduction pathway present a model organism that can be used to study the simultaneous biological and abiotic reduction of reactive intermediates such as NO2-. Additionally, Strain FeAm09 is the first isolated organism capable of autotrophic nitrate-dependent Mn(II) oxidation, thus demonstrating a previously unrecognized biogeochemical couple between Mn and N.
Tellurate Enters Escherichia coli K-12 Cells Via the Sulfate Transporter Cyspuwa

Primary Author Block:
J. Goff, N. Yee; Rutgers, New Brunswick, NJ

Abstract Body:
Background: Tellurium is an emerging environmental contaminant due to its increasing usage in electronics—especially thin-film solar panel technologies. The main oxidized forms of tellurium found in the environment are tellurite [Te(IV)] and tellurate [Te(VI)] (1). Both oxyanions are highly toxic to microorganisms. Bacterial interactions with tellurite are well-characterized. In contrast, comparatively little is known about how tellurate exerts its toxic effects on bacteria. Bacterial sulfate transporters are known to non-specifically transport toxic oxyanions such as selenate and chromate into cells (2). This study seeks to determine whether the sulfate transporters of Escherichia coli can also serve as transporters of tellurate. Methods: The wild type E. coli K-12 strain was grown with increasing levels of sulfate to determine sulfate’s effect on the strain’s level of resistance to tellurate. Mutant strains carrying knock-outs of the CysZ sulfate transporter and transmembrane subunit of the CysPUWA sulfate transporter (cysW) were assessed for their ability to grow in the presence of increasing levels of tellurate compared to the wild type strain. Tellurate uptake from the growth media by the different strains was assessed by measuring soluble tellurium concentrations using ICP-OES. Results: Tellurate resistance increased as the wild type strain was grown with increasing levels of sulfate. Additionally, inactivation of the CysPUWA sulfate transporter increased resistance compared to the wild type strain (MIC of 500 μM vs. 200 μM, respectively). Inactivation of the CysZ transporter had no effect and the mutant strain had a similar level of resistance as the wild type strain. Inactivation of the CysPUWA transporter resulted in an 86% decrease in tellurate uptake from the media as compared to the wild type strain. Conclusions: Our findings indicate that tellurate can enter E. coli cells via the CysPUWA sulfate transporter. This transporter is known to non-specifically transport other oxyanions including chromate and selenate. In contrast, the CysZ transporter is believed to be specific to sulfate and is not known to transport other compounds non-specifically, consistent with our findings here that it does not transport tellurate.
Energy is Necessary, But Not Always Sufficient for Survival of Microbial Populations in the Environment

Primary Author Block:
A. E. Howells1, J. Leong1, T. Ely1, M. Santana1, K. J. Robinson1, K. Fecteu1, P. A. Canovas1, A. Cox2, A. T. Poret-Peterson3, E. L. Shock1; 1Arizona State Univ., Tempe, AZ, 2Montana Tech, Butte, MT, 3USDA-ARS, Davis, CA

Abstract Body:
Theoretical energy availability of redox reactions in the environment can be compared with microbial presence to evaluate and help explain cases where energetic supply in the environment does not meet microbial demand. In such cases, it is predicted that factors such as availability of electron acceptors and donors, chemical inhibitors, biological competition and toxins may be controlling microbial population distribution. To test these predictions, geochemical and biological samples were taken at 29 sites across a geochemical gradient resulting from mixing between serpentinization-reacted fluids (> pH 11) and surrounding surface water (~ pH 8) in the Oman Samail Ophiolite. Disequilibrium generated by mixing between these two fluid types has the potential to fuel microbial metabolisms such as aerobic methane oxidation, hydrogen oxidation using O2 as the electron acceptor and hydrogenotrophic methanogenesis [1]. The presence of microbial populations known to use these strategies was evaluated by 16S rRNA gene sequencing of DNA extracted from sediments using methods described in [2]. Water samples were analyzed for major ions, dissolved gases and trace elements. Energy for each reaction was calculated using methods described in [1]. 16S rRNA genes classified as proteobacterial methanotrophs are present and most abundant in systems that are energy-rich and O2-rich. However, they are not present in all systems of this type. A known competitive inhibitor of methanotrophs, NH3, is enriched in hyperalkaline fluids, resulting in a NH3 gradient. Methanotrophs are not present in systems with > ~20μm NH3. A 16S rRNA gene classified as Hydrogenophaga, a known hydrogen oxidizer, is broadly distributed and dominant in hyperalkaline, energy-rich, H2-rich systems. The abundance of this population drops in mixing and surface water systems. While these systems are O2-rich, they also have significantly higher alpha diversity than sediments surrounded by hyperalkaline fluids. In these systems, Hydrogenophaga may be outcompeted for H2. A 16S rRNA gene classified as Methanobacteria, a methanogen, is found exclusively in hyperalkaline systems that are energy-poor and H2-rich. Systems that are energy-rich for this reaction also have atmospheric O2 levels, which can be toxic to methanogens. This population is not found in systems with O2 levels > 60μm. These results demonstrate that while energy is available in the environment it is not always sufficient for survival. Combining sequencing, energetics and geochemical data make it possible to explain why.
Variations in Microbial Community Structure with Lithology and Geochemistry in Shallow Subsurface Alluvial Aquifer Sediments

Primary Author Block:
O. M. Healy, J. P. Westrop, S. Antony-Babu, P. J. Nolan, D. Pan, R. V. Kiat, K. A. Weber; Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract Body:
Microbiota can significantly influence the geochemistry of aquifers and play a role in nitrate reduction. From a region with elevated groundwater nitrate concentrations that serves as municipal drinking water source, we coupled variations in microbial community structure and abundance with both lithological facies and geochemistry in two alluvial aquifer sediment cores, each more than 50 m in depth. The two cores (5A and 5D), 0.6 km apart, were collected near Hastings, Nebraska, an upland agricultural area, using sonic drilling. The site geology consists of a series of aeolian loess deposits that overlie a series of undifferentiated alluvial sands and gravels, which in turn overlie a marine clay-shale that acts as the regional aquitard. Sediment reduction potentials suggest both cores are relatively oxic with small reduced zones throughout. Most Probable Number (MPN) enumeration revealed an abundant heterotrophic nitrate-reducing community ranging from 7.4x10^4 cells g^-1 (right above the water table) to 1.9x10^9 cells g^-1 (right below the water table), while groundwater nitrate increased with depth (2.01-5.98 mg/L) from the water table. In the deepest reduced zone (52.73 m depth) an increased abundance of microorganisms that couple nitrate reduction to iron oxidation (5.6x10^9 cells g^-1) corresponded with a spike in total and reduced iron and uranium and transition to the marine Niobrara sediment deposit. To link microbial community structure to geological facies, subsurface sediments were grouped into six geologic facies: I.) Soil zone, II.) Silty clay, III.) Fe-stained silty clay, IV.) Silty sand, V.) Poorly sorted sand and gravel, and VI.) Clay shale. These facies were chosen based on lithological and geochemical parameters likely to influence community structure such as grain size, sorting, trace metal content, and carbon content. Culture independent microbial community analysis, regions in the 16S rRNA, 18S rRNA, and ITS1 genes elucidate the bacterial and archaeal, eukaryotic, and fungal community members for each facies. Correlating microbial community structure and function, lithological, and geochemical characteristics of these sediments allows for the prediction of microbial community structure from geochemical and lithologic subsurface characteristics and contribute to drinking water quality.
Abstract Title:
Atribacteria Adaptations for Life in Methane Ice
Primary Author Block:
J. B. Glass, P. Ranjan, A. M. Johnson, F. J. Stewart; Georgia Inst. of Technology, Atlanta, GA
Abstract Body:
Deep subsurface sediments that contain methane hydrates host unique microbial assemblages dominated by members of the Atribacteria (Inagaki et al., 2006), a candidate phylum of low-GC, gram-negative, fermenting anaerobes commonly found in anoxic, methane-rich habitats (Nobu et al., 2015). Methane hydrate ecosystems may pose a dual challenge for microbes due to salt stress and ice formation. In this study, we analyzed Atribacteria metagenome-assembled genomes (MAGs) for metabolic capacities and potential adaptations to life in methane ice. Sediment core samples were drilled from ODP site 1244 on South Hydrate Ridge, offshore Oregon on the Cascadia convergent margin on ODP Leg 204 and stored at -80° until DNA extraction. Multiple-displacement amplified DNA was used to generate genome libraries, sequenced by Illumina HiSeq, assembled into contigs, and partitioned into MAGs using MetaBAT. Protein-coding genes were annotated using Prokka and RAST. We focused our analysis on a MAG (B2, 69% completeness, 2% contamination) belonging to the Atribacteria JS-1 genus 1 lineage and recovered from the gas hydrate stability zone (~70 meters below the seafloor). Diverse adaptations to cold and osmotic stress, such as biosynthesis of compatible solutes, capsular polysaccharides, and “salt-out” strategies, were present in B2. B2 also displayed a wider range of metabolic capabilities than other Atribacteria, including amino acid fermentation, glycine betaine catabolism, and an uncharacterized electron transport chain. This study expands the known catabolic and anabolic repertoire of Atribacteria by showing that they may contain the genetic capacity for anaerobic respiration and survival mechanisms for osmotic stress. Ongoing research is exploring B2’s potential to encode ice antifreeze binding proteins due to its numerous outer membrane adhesin-like proteins.
Abstract Title:
Aerobic Respiration on Soluble Iron is Expressed Constitutively by Sulfobacillus Thermosulfidooxidans

Primary Author Block:
O. G. Griswold, N. T. Pham, R. C. Blake, II; Xavier Univ., New Orleans, LA

Abstract Body:
Very little is known about the electron transfer reactions that occur during aerobic respiration on soluble iron by members of the Firmicutes, a phylum of Eukaryotes that contains Sulfobacillus thermosulfidooxidans. This project was conducted to test the Hypothesis that iron oxidation is expressed constitutively in this moderately thermophilic, Gram-positive organism. Methods: Sb. thermosulfidooxidans was cultured organotrophically on yeast extract at pH 1.9 and mixotrophically on yeast extract and soluble ferrous iron at pH 1.5, both at 48°C. The time courses of ferrous iron oxidation as catalyzed by intact cells of Sb. thermosulfidooxidans were monitored using an integrating cavity absorption meter (ICAM) that permitted the acquisition of accurate absorbance data in suspensions of intact cells that scatter light. Initial velocities of iron oxidation were determined from the linear increases in absorbance at 350 nm due to the generation of oxidized iron. Results: Regardless of the immediate growth history of the organism, Sb. thermosulfidooxidans was observed to readily oxidize soluble iron and the kinetics of aerobic respiration on soluble iron by the intact organism conformed to the Michaelis-Menten formalism. It was thus evident that the ability to respire aerobically on iron was expressed in this organism under both growth conditions, which was consistent with the hypothesis. Electron transfer reactions among colored cytochromes in the intact eukaryotic cells were subsequently monitored in the ICAM in the presence of much higher concentrations of the intact cells. When whole cells were mixed with soluble iron at pH 1.5, the aerobic iron respiratory chain of Sb. thermosulfidooxidans was dominated by the redox status of one abundant cellular chromophore that had maximum absorbance peaks at 444 and 604 nm in the reduced state. The intracellular chromophore was reduced within the time that it took to mix a suspension of the intact bacterium with soluble ferrous iron. The reduced chromophore then returned to its original oxidized state as the organism catalyzed the complete oxidation of the limiting concentration of ferrous iron by the excess concentration of molecular oxygen. Conclusions: The kinetic behaviors of this reduced chromophore was consistent with the hypothesis that its oxidation represented the rate-limiting step in the overall aerobic respiratory process.
Examining the Impact of A Co2 Enhanced Oil Recovery Flood on the Microbial Community in the Target Oil and Gas Reservoir

Primary Author Block:
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Abstract Body:
Injecting CO2 into the deep subsurface to extract additional crude oil from depleted oil reservoirs is a common enhanced oil recovery (EOR) technique. However, little is known about how the microbial communities residing in these formations may be impacted by the CO2 flood, or if any permanent ecological changes occur to the formation after flooding has ceased. In this study, formation water was collected from various regions of an oil field that was flooded with CO2 for EOR in the 1980s (samples collected approximately 35 years later). Two water samples were collected from the area of the oil field that was impacted by the CO2-EOR flood, while 7 water samples were collected from areas that were outside of the flood region. Biomass was collected from the water by 0.22 µm filtration, DNA was extracted and amplified, and 16S rRNA gene sequencing was performed using the Illumina MiSeq platform. These two sample groups were compared in order to determine if the CO2 flood impacted the microbial community in this reservoir, or if the reservoir was able to “reset” back to pre-flood conditions (using the non-impacted samples as an analogue for pre-flood conditions). The dominating microbial communities of both groups was very similar: Archaea, specifically methanogens, dominated both the CO2-impacted and non-impacted samples while the identified Bacteria in all samples exhibited much more diversity than the Archaea. An anosim test comparing the CO2-impacted to the non-impacted samples revealed that the two groups’ microbial communities were not significantly different from each other (statistic R = -0.2597, significance = 0.769). However, several Bacteria were found to be significantly associated with the CO2-impacted group (p values between 0.024 and 0.046). Very few of these species are known to metabolize CO2 or are reported to be associated with CO2-rich habitats. Therefore, although these data cannot confirm whether or not the microbial communities in this reservoir were once impacted by the injected CO2, these results suggest that reservoirs impacted by a CO2 flood (or perhaps a CO2 leak) have the ability to rebound back to their pre-flood microbial composition.
Abstract Title:
Cryptoendolithic Bacterial Communities in the Sandstones of the Colorado Plateau Produce Exopolysaccharides that Sequester Metal Cations

Primary Author Block:
S. Kaur, H. D. Kurtz, Jr.; Clemson Univ., Clemson, SC

Abstract Body:
In the Navajo sandstone formations of the Colorado Plateau, a phenomenon known as “Iron-bleaching” has been studied previously. This process is largely attributed to the paleo geochemical processes that removed iron (III) from the hematite cladding of the sand grains within the stone, and resulted in the lightening of the stone. However, this process may still be occurring partly due to the action of the cryptoendolithic communities found in the sandstones of the region. Support for this hypothesis comes from previous research that showed the presence of δ-Proteobacteria associated with iron reduction. Further support comes from work that demonstrated the ability of extracellular polymeric substances (EPS) produced by cryptoendoliths to bind iron(II), which also explained the presence of readily detectable iron(II) in these ecosystems. Here we present additional data in support of our hypothesis. An analysis of the cryptoendolithic microbial community structure using Illumina Miseq sequencing analysis was conducted. Results revealed that Cyanobacteria were dominant, with Leptolyngbya, Chroococcidiopsis and unclassified cyanobacteria accounting for the bulk of cyanobacterial sequences. α-Proteobacteria were the next largest group detected, with members of the Acetobacteriaceae, particularly the genus Acidiphilium, being the most prevalent. Acidiphilium spp. are capable of aerobic ferric iron reduction under moderately acidic conditions, providing another explanation for high levels of iron(II) in these habitats. Analysis of the properties associated with EPS isolated from laboratory based communities and semi-purified cyanobacterial cultures using ICP spectroscopy, indicates that these polymers are capable of binding 22.5 nanograms of iron(II) / milligram of EPS. Competitive metal binding experiments suggest that the EPS has a role in binding other metal cations such as copper, zinc, manganese and magnesium. Based on these findings, we conclude that EPS produced by cryptoendoliths acts like a bio-filter concentrating nutrients and metal cations essential for the survival of microbial life in the sandstones.
Abstract Title:
Genome and Transcriptome Analyses of Staphylococcus aureus Forc_062, A Food-Borne Pathogen Isolated from Human Blood
Primary Author Block:
A. Cho, H. Chung, H. Im, D. Ko, S. Choi; Seoul Natl. Univ., Seoul, Korea, Republic of
Abstract Body:
Staphylococcus aureus is an ever-present worldwide public health threat and causes a range of diseases in human such as pneumonia, toxic shock syndrome and sepsis. S. aureus FORC_062 is a clinical isolate from human blood in South Korea and belongs to MRSA. To study FORC_062 at the genomic level, its whole genome was sequenced by PacBio. The genome consists of a circular chromosome of 2,905,353 bp with a GC content of 32.92%. The chromosome contains 2,727 open reading frames, 60 rRNAs, and 16 tRNA genes. Genes encoding many virulence factors were found by BLAST such as autolysin, α-, β-, γ-hemolysin, leukotoxin, toxic shock syndrome toxin. Average nucleotide identity analysis of the genome with 20 other whole genomes of S. aureus showed that FORC_062 is the most closely related to N315, a clinical isolate of a Japanese patient. Comparative genome analysis of FORC_062 and N315 revealed that FORC_062 has additional virulence factors such as autolysin N-acetylmuramoyl-L-alanine amidase and pathogenicity island SaPI. This may explain the high cytotoxicity of FORC_062 toward INT-407 human epithelial cells as determined in lactose dehydrogenase (LDH) release assay. To figure out the transcriptional response when the strain FORC_062 was expose to raw chicken breast, RNA sequencing was conducted. When FORC_062 contacted to chicken, genes related to amino acid permease and transporter were up-regulated, while genes related to amino acid biosynthesis were down-regulated. In addition, genes related to deamination, such as alanine dehydrogenase, threonine dehydratase were up-regulated. The results hinted that S. aureus may use the chicken as a reservoir to survival. All results described above demonstrated that S. aureus FORC_062 is high pathogenic to human and uses the chicken breast as a reservoir to survive and grow. More research is imperative to further our understanding of this important pathogen and to prevent future outbreak.
Abstract Title:
Transcriptome Profiles of Clin. Isolates Salmonella enterica in Contact with Fresh Cabbage, Lettuce, and Perilla Leaves

Primary Author Block:
J. Lee1, S. Kim2, R. B. Guevarra1, J-H. Lee3, H. Yoon2, H. Kim1; 1Dankook Univ., Cheonan, Korea, Republic of, 2Ajou Univ., Suwaon, Korea, Republic of, 3Kyung Hee Univ., Youngin, Korea, Republic of

Abstract Body:
Background: Salmonella enterica is one of the leading causes of food borne disease in the world. Recently, many cases of Salmonellosis outbreaks are caused by Salmonella-contaminated fresh vegetables, and one of the most common Salmonella serotypes associated with food borne diseases is Enteritis. Methods: For better understanding of the systemic gene regulations of S. enterica in contact with raw vegetables, S. enterica serotype Enteritis isolated from the feces of infected patients in South Korea were inoculated onto cabbage, lettuce and perilla leaves in a minimal medium broth for 2.5 hours. The bacterial mRNA was sequenced using RNA-Seq chemistry. All the experiments were duplicated. Results: Comparative transcriptome analysis of S. enterica serotype Enteritis in contact with fresh vegetables revealed that the nitrate-responsive two-component system NarX-NarL genes were significantly up-regulated. In addition, significantly up-regulated genes include those associated with flagella assembly, chemotaxis, and T3SS. High concentration of nitrate is often found on vegetables that are grown close to the ground because of nitrate fertilizer used to grow vegetables. Therefore, up-regulation of the nitrate-responsive two-component system NarX-NarL genes can be beneficial to bacteria growing and colonizing fresh produce. Also, up-regulations of genes associated with motility, chemotaxis, and T3SS has been known that they are required for bacteria to colonize vegetables through open stomata. Conclusions: Even though further studies are needed to elucidate roles of genes identified in this study during the process of Salmonella colonization in vegetables, results from this study suggest that the nitrate-responsive two-component system NarX-NarL and genes associated with motility, chemotaxis, and T3SS of S. enterica can be potential targets for genetic regulations to reduce Salmonella colonization in vegetables.
Abstract Title:
Characterizing the Microbiome in Factory Ingredient Samples Using Metatranscriptome Deep Sequencing Data

Primary Author Block:
K. L. Beck1, N. Haiminen2, D. Chambliss1, S. Edlund1, M. Kunitomi1, R. Baker3, P. Markwell3, M. Davis1, L. Parida2, R. Prill1, C. Huang4, N. Kong4, B. Kawas1, T. Marlowe5, S. Binder5, G. Dubois1, J. Kaufman1, B. Weimer4; 1IBM Res., San Jose, CA, 2IBM Res., Yorktown Heights, NY, 3Mars Inc, McLean, VA, 4UC Davis, Davis, CA, 5Bio-Rad Lab., Hercules, CA

Abstract Body:
Background: The microbiome of food ingredients can reveal characteristics that culture-based isolate sequencing alone cannot. We can monitor the food microbiome to understand food safety hazards and quality issues that may arise in the supply chain. For effective application of this, robust bioinformatics methods are necessary for accurate microbial identification. Additionally, food microbiome monitoring uniquely requires steps to authenticate the food matrix or host. Methods: The Consortium for Sequencing the Food Supply Chain presents a novel bioinformatics pipeline suited for these requirements as well as the validation methods applied to establish the accuracy for food testing. We also showcase a web application for the processing and analysis of these sample times. This pipeline has been applied to a collection of 31 poultry meal metatranscriptomes as part of monitoring a normal factory raw ingredient baseline. Results: We observe between 460 and 751 microbial genera per sample. The core poultry meal metatranscriptome appears remarkably stable across batches sampled from different vendors during a one year period. Bacteroides, Clostridium, Lactococcus, Aeromonas, and Citrobacter contribute the most variation to the consensus microbial genera and are also the most abundant. When sampling the “normal baseline,” we also observe samples with unexpected food matrix content (non-poultry meal). This content would not have been detected without shotgun sequencing of these food samples. Samples with unexpected matrix content are shown to have a higher proportion of microbial reads and increased abundance of certain genera and their respective family, order, class, and phylum. In addition to detecting known undesired microbes, such changes in the overall microbiome community composition could be used to detect potential food safety issues. Conclusions: By surveilling the microbiome of food ingredients, we can develop methods and best practices that can be used to improve food testing standards and security of the food supply chain.
Abstract Title:
The Role of Listeria Monocytogenes FruR Encodes Deor Transcriptional Regulator Factor in Virulence, Intracellular Replication, and Environmental Adaptation

Primary Author Block:

Abstract Body:
Listeria monocytogenes is a foodborne pathogen with a high mortality rate. It can survive in a variety of environmental stresses, including low pH, high salt, and low temperature. There is a knowledge gap in the regulatory mechanisms controlling metabolic adaptations of L. monocytogenes in stressful environments present inside and outside of host cells. In other bacterial species, members of the DeoR-family serve as transcriptional repressors or activators in sugar metabolism. The function and physiologic roles of DeoR-family regulators in L. monocytogenes have not been defined yet. We found seven members of DeoR-family regulators in the L. monocytogenes strain F2365 genome. We constructed a mutant strain by targeting FruR-encoding DeoR-family regulator (LMOf2365_2307). The virulence of F2365ΔfruR was studied in mice and macrophages cell lines. We found that F2365ΔfruR mutant is attenuated in mice. Also, F2365ΔfruR displayed significantly decreased invasion and replication in murine macrophages. The growth rate of F2365ΔfruR was lower than wild-type under salt (5% NaCl in BHI) and glucose (50 mM in minimal medium). Furthermore, F2365ΔfruR mutant showed high upregulation of phosphofructokinase (fruK) and fructose-specific transporter subunit IIABC (fruA) (50-100 fold, respectively) compared with the wild-type strain. Understanding the mechanisms allowing L. monocytogenes survival within host cells and stressful food environments will assist in the development of intervention strategies to control Listeria in food products and foodborne illnesses.
The Transcriptional Terminator of PorA Enhances Expression of the Major Outer Membrane Protein in Campylobacter jejuni

Primary Author Block:
L. Dai, Z. Wu, Q. Zhang; Iowa State Univ., Ames, IA

Abstract Body:
The porA gene in Campylobacter jejuni, a leading cause of foodborne illness worldwide, encodes the major outer membrane protein (MOMP) that is abundantly expressed and has important physiological functions. Recently, sequence polymorphism in MOMP is found to be responsible for hypervirulence of C. jejuni clone SA, which causes systemic infection and abortion in animals. Despite the importance of porA in C. jejuni pathogenesis, mechanisms modulating its expression levels remain unknown. Therefore, in this study we investigated the effect of the rho-independent transcriptional terminator for porA (TporA) on the expression of MOMP and virulence of Campylobacter. C. jejuni 11168 constructs containing the porA gene from IA3902 (a clone SA isolate) with either an intact TporA or an interrupted TporA were generated and compared for MOMP expression by Western blotting. Additionally, two GFP expression systems containing the porA promoter and GFP-coding sequence with or without TporA were constructed in Campylobacter. The two GFP constructs were subsequently compared for levels of gene transcription by qRT-PCR and protein expression by fluorescence measurement. To investigate the effect of TporA on Campylobacter virulence, the two C. jejuni 11168 constructs were examined for their ability to induce abortion in the guinea pig model. The immunoblotting results showed that the amount of MOMP protein was apparently lower in the C. jejuni construct with an interrupted TporA than in the one with an intact TporA. Transcriptional analysis by qRT-PCR showed that transcription of the GFP gene with TporA was ~2.4 fold higher than the GFP without TporA. Similarly, 2.2-2.4-fold increases in fluorescence level were detected in C. jejuni expressing GFP with TporA compared with C. jejuni expressing GFP without TporA. Guinea pig study showed that the C. jejuni construct with an intact TporA induced abortion in 75% of the inoculated animals, while the construct with an interrupted TporA only produced 25% abortion, indicating the level of MOMP production plays an important role in Campylobacter virulence. Together these results clearly demonstrate that TporA enhances the transcript level of porA and production of MOMP in C. jejuni and that the expression level of MOMP influences the virulence of Campylobacter.
Abstract Title: Identification of Protein Biomarkers for Genetic Lineage III Listeria Monocytogenes

Primary Author Block:

Abstract Body:
Listeria monocytogenes is the causative agent of listeriosis, a severe foodborne illness characterized by septicemia, meningitis, encephalitis, abortions, and occasional death in infants and immunocompromised individuals. In the U.S., L. monocytogenes is estimated to cause 1600 cases and about 260 deaths each year. L. monocytogenes is composed of four genetic lineages (I, II, III, and IV) and at least twelve serotypes. The aim of the current study was to identify proteins that can serve as biomarkers for detection of genetic lineage III strains based on simple antibody-based methods. Through 2-DE gel proteomic analysis and liquid chromatography (LC) with electrospray ionization tandem mass spectrometry (ESI MS/MS) analysis, we identified 19 distinct proteins in lineage III strain ST 33077 that have no homologs in representative strains from genetic lineages I and II. BLAST analysis of the 19 proteins against a broader panel of >80 sequenced strains from lineages I and II revealed six out of these nineteen proteins have no identity with any sequenced strains in lineages I and II. The six genes that encode these proteins (ST33077_2218, ST33077_2323, ST33077_2770, ST33077_1897, ST33077_1926, and ST33077_1129) were amplified from L. monocytogenes 33077 and cloned into expression vector pET28a. Four proteins out of six were expressed in E. coli strain DE3 and purified by His-Bind resin. The purified recombinant proteins (ST33077_1897, ST33077_2770, ST33077_1926, and ST33077_1129) have estimated molecular weights of 21.09, 20.9, 33.6 and 107 kDa, respectively. These recombinant proteins have potential for development of polyclonal/monoclonal antibodies specific for L. monocytogenes lineage III and demonstrate the feasibility of this approach for using protein biomarkers to distinguish L. monocytogenes genetic lineages. Development of a protein-based detection method for distinguishing L. monocytogenes lineages would facilitate their identification in diagnostic microbiology laboratories and in food processing facilities. This increased discriminatory capability would assist in assessment of risk from L. monocytogenes isolates and accelerate epidemiological investigations.
Abstract Title: Outstanding Abstract Award: Emerging IVb-v1 Clone (CC554) of Listeria monocytogenes is Highly Prevalent among Strains from Suburban Black Bears (Ursus americanus)

Primary Author Block: C. Parsons1, Y. Chen2, Z. Kucerova3, S. Kathariou1; 1North Carolina State Univ., Raleigh, NC, 2Ctr. for Food Safety and Applied Nutrition, Food and Drug Admin., College Park, MD, 3CDC, Atlanta, GA

Abstract Body: Listeria monocytogenes is a facultative intracellular pathogen responsible for the foodborne disease listeriosis. While cases of listeriosis are uncommon, the severity of symptoms which include septicemia, stillbirths, meningitis or even death in susceptible individuals render it a major cause for public health concern. One of the reasons that L. monocytogenes proves to be so problematic is its wide distribution in the environment, having been isolated from such diverse sources as soil, water, vegetation and various animals. To better elucidate the ecology of this pathogen, and examine how strains may be interfacing between humans and wildlife, we recently conducted a survey of L. monocytogenes prevalence among black bears (Ursus americanus) in the Southeastern US, mostly in urban and suburban North Carolina. This survey was conducted over three years (2014-2017) in conjunction with a larger wildlife conservation study. Selective enrichments were performed on feces, nasal and rectal swabs, and 104 of the isolates were analyzed by whole genome sequencing. In silico serotype analysis indicated that a substantial portion (39%) were serotype 4b, one of the three L. monocytogenes serotypes most commonly associated with human disease. Almost half (45%) of the serotype 4b strains were found to belong to the IVb-v1 serotype 4b subgroup. Multilocus sequence typing revealed that most of these IVb-v1 strains belonged to clonal complex (CC)554 (mostly ST554), making it by far the most predominant CC. CC554 was detected through all three years of the survey and was isolated from bears in N. Carolina and Virginia suggesting that its prevalence is both stable and geographically diverse. CC554 appears to be an emerging CC in North America and was implicated in a produce-associated outbreak in the US in 2014. Analysis of L. monocytogenes from human listeriosis in N. Carolina during 2014-2017 revealed four that were CC554. These findings suggest that black bears may prove to be an important wildlife host for L. monocytogenes, potentially playing a role in the evolution and dissemination of novel clones of this pathogen.
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Session Type: Poster
Session Title: AES17 - Foodborne Pathogens: Genetic, Genomic, and Microbiome Studies
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 3953
Poster Board Number: SATURDAY - 933

Abstract Title:
Baseline Microbiomes and Resistomes in Selected Animal Food

Primary Author Block:
Q. Yang1, D. A. Tadesse1, K. J. Domesle1, K. G. Jarvis2, C-H. Hsu1, S. H. Sarria1, X. Li3, B. Ge1;

Abstract Body:
Animal food, e.g., pet food, animal feed, and raw materials and ingredients, is a diverse and complex food matrix. High-throughput sequencing facilitates the characterization of the microbiota and antimicrobial resistance genes associated with these matrices. In this study, we used 16S rRNA gene amplicon and shotgun metagenomic sequencing to profile the microbiomes and resistomes of representative animal foods (cattle feed, poultry feed, and dry dog food). Additionally, a mock microbial community was included for method evaluation. Four genomic DNA extraction methods were compared and 16S rRNA gene amplicon (targeting three regions) and shotgun metagenomic DNA libraries were sequenced on Illumina’s MiSeq and NextSeq platforms, respectively. Quality-filtered 16S rRNA gene sequences were analyzed using multi-step OTU picking by QIIME. Taxonomic and resistome profiles of shotgun metagenomic sequences were determined by MetaPhlAn2 and ShortBRED, respectively. Both 16S rRNA gene and shotgun metagenomic sequencing data suggested that ZymoBIOMICS DNA miniprep kit provided taxonomic profiles most resembling the theoretical values of the mock community and the V3-V4 region of the 16S rRNA gene was the most accurate. Shotgun metagenomic sequencing revealed distinct microbiota among the animal foods tested. At the genus level, the most abundant taxa among all animal foods were Pantoea (35.4%) followed by Xanthomonas (21.7%), Pseudomonas (15.0%), and Bacillus (11.4%). Salmonella was observed in one cattle feed and two poultry feed samples at relative abundances below 5%. A total of 28 resistance genes conferring resistance to 8 antimicrobial drug classes were identified. Animal feed samples harbored resistance genes from all 8 classes while pet food only contained genes conferring resistance to β-lactams and trimethoprim. These data enhance our understanding of endemic microbiota as well as resistance genes present in animal food. Such information may be useful in future microbial risk assessment efforts to enable better characterization and control of microbiological hazards in animal food.
Abstract Title:
Reduction of Salmonella and Shiga-Toxin-Producing Escherichia coli (Stec) on the Surface of Alfalfa Seeds and Sprouts by Combined Antimicrobial Treatments Using Ozone and Electrolyzed Water

Primary Author Block:
Z. H. Mohammad; Texas A&M Univ., College Station, TX

Abstract Body:
Many foodborne outbreaks have been associated with consumption of raw alfalfa sprouts. Salmonella and Shiga toxin-producing E. coli (STEC) have been the most common pathogens implicated in outbreaks of foodborne illness linked to contaminated alfalfa sprouts. The seed has been identified as a major source of contamination. Individual chemical and non-chemical treatments have failed to completely eliminate pathogens from alfalfa seeds and sprouts. This study investigated the disinfection of alfalfa seeds and sprouts using an intervention combining ozone with electrolyzed water treatments. Alfalfa seeds inoculated with a cocktail of 3 strains of Salmonella and 3 strains of STEC were treated sequentially with aqueous ozone followed by acidic (pH 3.0) electrolyzed water. The samples of inoculated seeds or sprouts were first immersed into 1 L of ozonated water (5 mg/L ozone) for 15 or 20 minutes with continuous oxygen feeding pressurized with 10 psi. The samples of inoculated seeds or sprouts then were immersed in 1 L of acidic electrolyzed water (EW) for 15 min. Salmonella and STEC were significantly (P <0.05) reduced on the seeds and sprouts. Mean log reduction were 3.6±0.3 and 2.9±0.2 log CFU/g, respectively for seeds, and 3.1±0.2 and 3.0±0.1 log CFU/g, respectively for sprouts. There were no differences (P >0.05) in the magnitude of the log reduction between Salmonella and STEC on seeds and sprouts or between seeds and sprouts. The effect of ozone and EW treatments on the quality of sprouts including shelf life, weight and color was also evaluated. The results showed no significant changes in these parameters on treated and non-treated sprouts. It was concluded that the combination of ozone and EW was effective in inactivating Salmonella and STEC on the surface of alfalfa seeds and sprouts with no negative effects on the quality and color of sprouts. Keywords: Salmonella, STEC, Electrolyzed water, Sprouts, Alfalfa
Abstract Title:
Yeast As An Expression Platform for Endolysin
Primary Author Block:
J. Chun, J. Bae, S. Ryu; Seoul Natl. Univ., Seoul, Korea, Republic of
Abstract Body:
For several decades, strategies to substitute conventional antibiotic agents have been extensively studied in the field of food science. Among them, bacteriophage has been recognized as an effective option to control foodborne pathogenic bacteria. Specifically, endolysin, a lytic protein encoded by bacteriophage, has been studied as a novel biocontrol agent. However, despite the advantages, under the conventional bacterial expression platform, there exist several limitations in protein harvest, extraction or refinement. In this research, yeast surface display system was employed to resolve aforementioned limitations and to suggest an effective antibacterial strategy. As a target host, Staphylococcus aureus ATCC 13301 was selected, and endolysin LysSA11 from phage SA11 was selected as model antibacterial agent. LysSA11 gene was ligated into a shuttle vector pCTCON at the downstream of yeast anchor protein Aga2a. The construct was initially cloned into Escherichia coli DH5α and then to the expression host, Saccharomyces cerevisiae EBY100. The clones were confirmed successful by PCR and sequencing. Selected clones were cultivated in synthetic media with 2% glucose and transferred to synthetic media with 2% galactose for induction at 30°C, 250 rpm. According to western blot assay, Aga2a-LysSA11 was efficiently expressed within the cell. By flow cytometry, it was learned that LysSA11 was displayed in over 50% of yeast population. To figure out the optimal induction period, endolysin-displaying yeasts were harvested at 12 h, 16 h, 20 h post induction for lytic activity test. For each case, 4x10^8 CFU/mL of yeast and 4x10^3 CFU/mL of S. aureus were combined in reaction buffer and incubated for 4 h at 25°C. As a result, yeast with 16 h induction exhibited superior activity by totally eliminating 3.62 log CFU of S. aureus in less than 3 h. When the yeasts were induced for 20 h, the lysis efficiency decreased to 2.03 log reduction during 4 h reaction. On the other hand, no lytic activity was observed with 12 h-induced yeast samples. The yeast surface display system allowed a simple and efficient expression of phage derived antibacterial agent with promising lytic activity against S. aureus. It is suggested that yeast display platform may change the labor-intensive nature of endolysin production and be employed to various antibacterial applications for food safety.
**Abstract Title:**
Immunological Reactions to Salmonella Flgk and Flid Flagellar Proteins by Broiler Sera

**Primary Author Block:**
H-Y. Yeh1, A. Acosta2, K. Serrano2; 1USDA/ARS/PMSPRU, Athens, GA, 2Univ. of Puerto Rico, Mayaguez, PR

**Abstract Body:**
Background: Salmonella is the leading foodborne pathogen that causes human acute bacterial gastroenteritis worldwide. Chickens are considered as one of major reservoirs of this bacterium. Because the bacterial flagellum is involved in motility, adhesion, quorum sensing and other virulence activities, the flagellum may be the targets for immune responses by chickens. The flagellum is composed of more than 35 proteins. Two flagellar proteins - FliD and FlgK- were selected for the study due to their exposure to the environment and involvement in colonization in the intestinal mucosa. This communication describes expression and characterization of these two proteins, and their antigenicity of the FliD and FlgK proteins in chickens. Methods: The fliD and flgK genes were amplified by PCR, and the proteins were over-expressed in an Escherichia coli Expression System. The recombinant proteins were purified by a nickel-chelating affinity chromatography, and confirmed by SDS-PAGE analysis, the His tag detection and MALDI-TOF analysis. The recombinant proteins was tested for their antigenicity with ELISA using chicken sera from several geographical locations. Results: The recombinant FliD and FlgK proteins were purified by a His-tag affinity chromatography and had a respective, relative mobility of relevant sizes and positions in SDS-PAGE. Sera from the FlgK and FliD immunized broilers reacted strongly to FlgK and FliD, respectively, indicating that these proteins are immunogenic. Further, we used FliD and FlgK as probe to survey prevalence of anti-Salmonella antibodies in broilers. The ELISA showed 66% of broiler sera reacted to FlgK, while about 38% to FliD. The results implicating that these anti-FlgK antibody may be prevalent in the poultry population. Conclusion: These results provide a rationale for further evaluation of these proteins as vaccine candidates for broiler chickens so that food safety for poultry can be improved. These proteins may also hold important insights for Salmonella commensalism in chickens and pathogenesis in humans.
Abstract Title:
Hepatitis A Virus Inactivation on Formica Coupons by Chlorine Dioxide Gas

Primary Author Block:
A. Bowman, M. Morgan, D. Lockwood, D. D'Souza; Univ. of Tennessee, Knoxville, TN

Abstract Body:
Background: Hepatitis A virus (HAV) outbreaks have been increasingly reported in the United States. Improved food processing and decontamination methods are being researched to prevent their spread. Chlorine dioxide (ClO2) gas is a strong oxidizing agent with antimicrobial activity over a broad pH range (3 to 7). Chlorine dioxide gas has shown improved effectiveness over chlorine solution against bacteria and some human noroviruses, with increased penetration abilities. In addition, halogenated by-products with humic substances are not formed with ClO2 gas. The objective of this research was to determine the ability of ClO2 gas to inactivate HAV on formica coupons at room temperature (RT).

Methods: HAV (~ 6 log PFU) with 8% fetal bovine serum (FBS) to simulate organic load or without FBS was aseptically dried on sterile formica coupons in a biosafety cabinet. These coupons were then treated with 8 mg/L ClO2 gas for 0, 2, 3, 5, 8, 10, 12, and 15 min at ~80% relative humidity and RT. After each treatment-time, HAV was eluted using cell-culture media containing 8% FBS, serially diluted in cell-culture media containing 2% FBS and plaque assayed using confluent FRhK-4 cells in 6-well plates. Each assay was carried out in duplicate and replicated thrice, and data were analyzed.

Results: HAV titers without organic load were decreased by ~2.24, 2.11, 2.36, 2.41, 2.96, 3.69, and 4.13 log PFU by 8 mg/L ClO2 gas after 2, 3, 5, 8, 10, 12, and 15 min, respectively. HAV titer reduction with organic load on coupons was similar to HAV without organic load, where decreased titers of ~2.16, 2.27, 2.40, 2.46, 2.51, 4.14, and 5 log PFU after treatment with 8 mg/L ClO2 gas for 2, 3, 5, 8, 10, 12, and 15 min, respectively were obtained.

Conclusions: Increased reduction in HAV titers of ~4 to 5 log PFU on formica coupons can be obtained with treatment of 8 mg/L ClO2 gas for 12 and 15 min, and with lower reduction of ~2 log PFU from 2 to 8 min treatment with 8 mg/L ClO2 gas. Thus, higher concentration of 8 mg/L ClO2 gas and longer treatment times of 12 to 15 min show promise for environmental application to decrease HAV titers on contact surfaces and to prevent HAV transmission and outbreaks.
Abstract Title:
Antibacterial Activity of the Non-Glycoprotein Fraction of Melipone Beecheii and Apis mellifera Honey against Foodborne Pathogens

Primary Author Block:
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Abstract Body:
Background: High incidence of acute diarrhea caused by microorganisms related to foodborne diseases has provoked the search for solutions to avoid this problem. An alternative is the use of regional honeys from Melipona beecheii & Apis mellifera, which have been determined to have an antibacterial potential against various pathogens related to Foodborne Illness like E.coli O157: H7, E. coli, S. aureus among others. We have reported the antimicrobial activity of Melipone and Apis honey and their total protein extract. However, there are no reports about the antimicrobial potential of specific proteins from these honeys, yet. The aim of this study was to generate knowledge of the honey proteins, determine the antibacterial potential of glycosylated and non-glycosylated honey proteins and their contribution to the antimicrobial activity of honey. Methods: Honey samples were collected from beehives in the state of Yucatan, Mexico. Strains used were E. coli ATCC 25922, E. coli O157: H7S and S. aureus 25923. The extraction of proteins from honey was carried out by ultrafiltration. The separation of the protein extract was carried out by affinity chromatography (concavalin A). The proteome of honey proteins was determined using SDS-PAGE and 2D system. Moreover, the proteolytic activity was determined using electrophoretic methods (zymogram). The antimicrobial effect was evaluated by disk diffusion method and the minimum inhibitory concentration (microdilution method). Results: The protein extract of Melipona showed antimicrobial activity against E.coli O157: H7, with a MIC value of 300 μg/mL. Total protein extract of Apis mellifera showed antimicrobial activity against both bacteria tested, with inhibition halos of 19 mm (E. coli) and 27 mm (S. aureus). The MIC value for E. coli was 3800 μg/mL and for S. aureus was 932 μg/mL. Proteome analysis of Melipona and Apis honey by 2D electrophoresis showed a total of 24 and 20 different proteins respectively. Antimicrobial analysis revealed that the non-glycoprotein fraction could inhibit the growth of bacteria tested; furthermore, this fraction has proteolytic activity by substrate-gel electrophoresis. Conclusions: Honey proteins contribute and have a direct correlation with the honey antibacterial activity. These proteins have antimicrobial activity against Gram positive and Gram negative bacteria. Non-glycosylated proteins fraction contain at least one protease that might be the main responsible compound for antimicrobial potential of these proteins.
Abstract Title:
L-Lysine Can Trigger Germination of Spores of Clostridium Perfringens

Primary Author Block:
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Abstract Body:
Clostridium perfringens type A isolates cause a wide spectrum of diseases in humans, including food poisoning (FP) and non-food-borne (NFB) gastrointestinal diseases. Spore germination is considered as the most essential step for initiation of these diseases. Germination is initiated when bacterial spores get contacted with specific nutrients called germinants. Previous studies have identified amino acids such as, L-cysteine, L-asparagine, L-serine, and L-threonine, all at pH 6.0, as germinants for spores of C. perfringens FP and NFB isolates. In this study, we showed that (i) L-lysine (pH 6.0) triggered germination of spores of all tested FP and NFB isolates; although extremely low concentration of L-lysine (5-10 mM) induced germination of FP spores, 10-fold higher concentration (50 mM) was required for NFB spore germination; (ii) NFB strain F4969 gerKC spores did not germinate, FP strain SM101 gerKC spores germinated extremely poorly and these gerKC spores released significantly less DPA as compared to wild type spores; and these defects were restored to a nearly wild-type level by complementing gerKC spores with wild-type gerKC; and (iii) F4969 gerAA spores also did not germinate, and released less DPA than wild-type spores in presence of L-lysine; and these defects were restored partially (germination) and fully (DPA release) by complimenting gerAA spores with wild-type gerAA. Collectively, our current study identified L-lysine as a universal germinant for spores of both FP and NFB C. perfringens isolates and provided evidence that GerKC (from SM101 or F4969) and F4969 GerAA play major roles in L-lysine-induced germination.
Abstract Title:
Defining Roles of Ycfr In Biofilm Formation In Salmonella Typhimurium 14028s

Primary Author Block:
S. Kim, H. Yoon; Ajou univ., Suwon, Korea, Republic of

Abstract Body:
Background: It’s been widely accepted that Salmonella food poisoning is caused mainly by contaminated meat and poultry products. However, a number of recent studies demonstrated that raw vegetables were also causative of Salmonella infection. Methods: Transcriptomic profiling of Salmonella spp. showed that ycfR was significantly induced in contact with raw vegetables including cabbage, suggesting an essential role of ycfR in Salmonella adaptation in plants Results: Gene ycfR is predicted to encode an outer membrane protein YcfR and reported to be important for resistance against diverse stressors in many different bacteria. Therefore, we aimed to characterize the roles of ycfR in Salmonella under diverse conditions. A mutant strain lacking ycfR showed growth rates and viability comparable to wild-type strain regardless of the presence of cabbage. However, the ΔycfR strain exhibited increased biofilm formation abilities on biotic and abiotic surfaces. In accordance with the increased biofilm formation, bacterial motility was decreased in the absence of ycfR, while the expression of curli fimbrial genes was increased. Furthermore, the lack of ycfR caused significant structural alterations in outer membrane components, including lipopolysaccharide (LPS) and outer membrane proteins. These structural changes increased bacterial hydrophobicity of cell surfaces, thereby leading to cellular auto-aggregation. Intriguingly, the ΔycfR strain was more permeable to hydrophobic fluorescent probe N-phenyl-1-napthylamine (NPN) and more susceptible to acid treatments, suggesting impaired cell integrity by the lack of ycfR Conclusions: These results indicate that ycfR is required for maintaining the integrity of the outer membrane of Salmonella.
Abstract Title:
Long-Term Survival and Thermal Death Kinetics of Enterohemorrhagic Escherichia coli O26, O103, O111 and O157 in Wheat Flour

Primary Author Block:
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Abstract Body:
Wheat flour has been considered a relatively safe food because of its low water activity (aw), but it was recently associated with gastroenteritis outbreaks caused by enterohemorrhagic Escherichia coli (EHEC). Since the occurrence of EHEC outbreaks linked to low aw foods has been very rare, their ability to survive and tolerate heat in flour is not known. This study was undertaken to assess the long-term survival and thermal death kinetics of EHEC in wheat flour. Flour samples were inoculated (8 Log CFU/g) with 5-strain cocktails of each serogroup and stored at room temperature (23 ºC) and 35 ºC for long-term survival experiments or used for thermal inactivation at 55, 60, 65 and 70 ºC. Decimal reduction times (D and δ values) were calculated using Log-linear and Weibull models. At room temperature, the viable count of the four serovars declined gradually and was quantifiable for 12 weeks post-inoculation with a 2 Log CFU/g limit of detection. Upon enrichment, positive samples for EHEC were still observed after 40 weeks. At 35 ºC, viability decreased rapidly and EHEC O26 and O157 serovars were not quantifiable after only a week and no positive samples were detected by enrichment after 4 and 7 weeks, respectively. At room temperature, D-values were approx. 8 days and δ-values were in the range of 3.1-5.3 days, but no difference among serogroups (p ≤ 0.05). Treatment of the flour at 55 and 65 ºC resulted in δ value ranges of 15.6- 39.7 min and 3.0 to 3.7 min, respectively, with no significant difference among serogroups, either. The Z- values were 12.6, 6.7, 10.2 and 13.4 ºC for O26, O103, O111 and O157, respectively. The thermal death kinetics of EHEC in flour were better described by using the Weibull model. Keywords: wheat flour, enterohemorrhagic Escherichia coli, long-term survival
Abstract Title:
Bacillus; Bacteriocin Producers As Biocontrol Agents for Staphylococcus aureus

Primary Author Block:
V. Chhetri; Faculty of Sci., Bangkok, Thailand

Abstract Body:
This research is aimed to isolate, bacteriocin producing halophilic bacteria from salty fermented foods, i.e. Plara (Thai traditional salty fermented fish) and soya sauce, with inhibitory activity against different strains of Staphylococcus aureus, to be implemented as bio-control agent. Bacterial communities of Plara and soya sauce samples were analysed by two methods, cultural dependent and cultural independent method (Reverse Transcriptase PCR DGGE (Rev-T PCR-DGGE)), and subsequently sequenced by 16s rRNA analysis. Halanaerobium spp. in Plara and Staphylococcus gallinarum in soya sauce were the main population detected by the Rev-T PCR-DGGE. On the other hand, Bacillus spp. were the main isolates detected by cultural plating method. Among 126 isolates from soya sauce and plara, 37 isolates (35 Bacillus strains and 2 other groups of bacteria) exhibited inhibitory effect against the three different indicator strains of Staphylococcus aureus. The inhibitory action was tested by deferred antagonism, spot-on-the-lawn method. The Bacillus isolates displayed different inhibitory pattern on the indicator strains, significantly different at p<0.05. The Bacillus isolates with positive inhibitory action was further investigated for the gene encoding bacteriocin production (subtilin-spa/ subtilosinA-sbo). Furthermore, bacteriocin gene expression was studied, and found that two isolates of Bacillus (B. amyloliquefaciens and B. pumilus) expressed bacteriocin gene in 5% NaCl and co-culture with S. aureus. Bacillus isolates expressing bacteriocin gene were selected for developing as dried starter culture and further determined their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) prior to application in food sample. The culture showed significant inhibitory effect on Staphylococcus aureus. Thus, this study demonstrates a potential of Bacillus spp. for further development as autochthonous bio-preservative in food systems. Key words: Bacteriocin, Bacillus, DGGE, MIC, MBC
Session Title: AES17 - Foodborne Pathogens: Physiology and Control
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 5800
Poster Board Number: SATURDAY - 943

Abstract Title:
Effective Inhibition of Salmonella Typhimurium in Fresh Produce Using A Phage Cocktail Targeting Multiple Host Receptors

Primary Author Block:
J. Bai, S. Ryu; Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract Body:
Salmonella contamination of fresh produce is the primary bacterial cause of a significant number of foodborne outbreaks and infections. Bacteriophages are considered as a natural antibacterial agent for the control of foodborne pathogens. However, the rapid development of bacterial resistance to phage infection is a significant barrier to practical phage application. To overcome this problem, we developed a novel phage cocktail consisting of the three phages (BSPM4, BSP101 and BSP22A) that target different host receptors, including flagella, O-antigen and BtuB, respectively. Whole genome sequence analysis of the phages revealed that three phages do not harbor genes involved in lysogen formation or virulence in their genomes, suggesting they are safe to be applied on foods. In vitro challenge assays and BIMs (Bacteriophage Insensitive Mutants) frequency analysis revealed that the three-phage cocktail showed a superior host growth reduction compared to the two-phage cocktails or the single phages. Phage cocktail treatments with an MOI (Multiplicity of Infection) of 103 to 104 achieved 4.7~5.5 log CFU/cm² reduction of viable cell number in iceberg lettuce and 4.8~5.8 log CFU/cm² reduction in cucumber, respectively, after 12 hours at room temperature. The phage cocktail exhibited good antimicrobial efficiency, suggesting that it could reduce S. Typhimurium contamination of fresh produce. The strategy for phage cocktail preparation based on receptor utilization described in this study can be applied to develop phage cocktails against other pathogens as well.
Session Number: 259
Session Type: Poster
Session Number: 259
Session Type: Poster
Session Title: AES17 - Foodborne Pathogens: Physiology and Control
Session Start Date Time: 6/9/2018 11:00:00 AM
Session End Date Time: 6/9/2018 1:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 7233
Poster Board Number: SATURDAY - 944

Abstract Title:
Serovars of Salmonella enterica Vary with Respect to Expression of Cell-Surface Components and Interactions with Red Leaf Lettuce

Primary Author Block:
A. N. Reid, K. Beaton, N. Donahue, H. Kennedy, B. LoCascio, C. Marsocci; Salve Regina Univ., Newport, RI

Abstract Body:
Recent years have seen an increase in the number of outbreaks of salmonellosis linked to the consumption of fresh fruits and vegetables. An understanding of the molecular mechanisms that contribute to S. enterica’s fitness in/on this commodity could lead to strategies to reduce the burden of salmonellosis. Attachment and colonization of fresh produce is likely to be mediated by molecules on the surface of the bacterium, such as polysaccharides and flagella. The aim of this research is to determine whether expression of cell-surface structures varies from one S. enterica serovar to another, and whether these differences correlate with a serovar’s ability to attach to, colonize and persist on leaf lettuce. We also seek to understand whether conditions used to prepare inocula for in vitro plant interaction studies influence the expression of cell-surface structures, and consequently influence the outcome of these studies. Selected serovars (Agona, Enteritidis, Javiana, Montevideo, Newport and Typhimurium) were grown in LB and low-salt LB plates, broth and biphasic cultures, at 25 and 37°C. Expression of cellulose and fimbriae was assessed by growth on media containing calcofluor white and Congo Red, respectively, while biofilm formation was measured in a microplate assay. Flagella were isolated and visualized on Coomassie-stained SDS-PAGE gels and Western immunoblots, while motility was determined by inoculation on semi-solid agar. Serovar-specific differences in flagellin and cellulose expression were detected, as were differences in degree of motility and biofilm formation. These variables were also influenced by inoculum preparation conditions, though not in a consistent manner across serovars. Serovars grown under the above conditions were spot-inoculated onto red leaf lettuce, recovered after 30s, 1h or 5d, and enumerated on XLD agar. With few exceptions, the expression of cell-surface structures in culture was not a good predictor of attachment, colonization and persistence on red leaf lettuce. The serovars showing the highest levels of interaction with the lettuce varied with attachment phase and with growth conditions used for inoculum preparation. Overall, these studies reveal tremendous variability in plant interaction assays, and highlight the impact of inoculum preparation conditions on these studies.
Attachment of Ehec to A Stainless-Steel Surface Under Different Environmental Conditions

Primary Author Block:
A. L. Kraft, T. M. Bergholz; North Dakota State Univ., Fargo, ND

Abstract Body:
During the pre- and post-harvest processing of fresh produce, the attachment of enterohemorrhagic Escherichia coli (EHEC) to food-contact surfaces is an important step in facilitating transmission to humans. In these environments, the bacteria encounter many varying types of conditions. To better understand how environmental conditions impact the attachment of EHEC to an abiotic surface, we utilized an attachment assay with strains from the two EHEC serotypes, O157 and O26, on stainless-steel coupons under the following test conditions at room temperature (25°C): phosphate buffered saline (PBS), glucose-defined minimal media (GDMM), 4.5% NaCl, and 4% lettuce lysate. Strains were grown to a concentration of 10^6 to 10^7 CFU/ml in GDMM and the assay was performed in a 6-well plate with a sterile stainless-steel coupon submerged in 7.2 ml of each test condition and 0.8 ml of the inoculum. At 15, 30, 60, and 90 min post inoculation, samples from the free media in the well, poorly-adhered cells, and adhered cells were collected from each well/coupon, diluted, and plated on LB for enumeration. Poorly-adhered cells were removed from the coupons by washing with PBS+Tween for 20 sec and adhered cells were removed using PBS, glass beads, and vortexing for 2 min. When comparing the poorly-adhered and adhered counts collected from coupons incubated in GDMM, salt, and lysate to those counts from PBS, we found that the O157 strain exposed to 4% lysate had nearly 4 times greater attachment while the O26 strain was about 2 times lower over the 75 min period. However, poorly-adhered cell counts were 1.5 times greater for O26 in lysate when compared to PBS. For the O157 strain, the salt condition lead to 2 times lower attachment of poorly-adhered cells when compared to that of PBS. Attachment, adhered and poorly-adhered, in GDMM was similar to PBS for both strains, and for O26 the attachment in the salt condition was similar to PBS as well. Overall, our data indicate differences in attachment between these two serotypes under different test conditions, and further work is needed to elucidate which surface structures involved in attachment are influenced by different environmental conditions.
Abstract Title:
Culturable Bacterial Communities of Romaine Lettuce Leaves and Application of A New Optical Scattering Technology (Beam) for their Characterization

Primary Author Block:
D. Sarria Zuniga, E. Bae, A. Deering, M. Aime, J. Robinson, R. Pruitt; Purdue Univ., West Lafayette, IN

Abstract Body:
Recent studies have demonstrated that Salmonella spp. and Escherichia coli O157:H7 can internalize within the plant tissues1-3. Romaine lettuce is susceptible to contamination by human pathogens which must compete with the resident bacteria for resources and adaptability in this ecological niche4. Therefore, the characterization and study of the roles of the bacterial communities of Romaine lettuce, can provide insights to protect against human pathogen invasion. For that reason, BEAM technology was trained to determine if can become a rapid and inexpensive method for classification of these bacterial communities. The culturable bacterial populations of Romaine lettuce leaves were determined from 24 heads (12 conventional and 12 organic) from grocery stores. Leaves were blended and isolated colonies on PCA were identified by Sanger sequencing of 16S. Then, these colonies were used to test the BEAM technology developed by Purdue, in which a 635-nm laser beam passes through the center of a colony and generates a scatter pattern that might be used for identification after building a pattern classification library5. Conventional lettuce leaves had lower bacterial populations (1.93 ± 0.59 x10^5 CFU/g) than organic ones (1.60 ± 0.77 x10^6 CFU/g). Also, organic lettuce (42 genera) presented higher diversity of the culturable bacterial genera than conventional lettuce (22 genera). Finally, 645 colonies were clustered into 69 OTUs with Pseudomonas and Arthrobacter as the most common in both types. To train BEAM for the characterization of bacterial communities, 30 strains representing 33% of total OTUs and 80% of total strains, were used to collect scatter patterns and build a classification library. Training sets split by the optimal time of incubation and with Positive Predictive Value (PPV) above 90% were created, this suggested that BEAM technology can differentiate bacterial genera isolated from romaine lettuce with up to 10% of overlap. To test the created libraries, pure and mixed cultures of the same strains used in the training sets were evaluated to determine their correct classification. The misclassification can be up to 40% due to the overlap among some classes. In conclusion, organic romaine lettuce leaves have higher culturable bacterial populations with more diversity of bacterial genera. Additionally, BEAM technology has the potential to differentiate bacterial genera and become a tool to characterize culturable communities. However, issues such as similar scatter patterns from some different bacterial genera need to be improved.
Abstract:
Rna-Seq Analyses on Listeria Monocytogene AndRalstonia Insidiosa Mono- and Dual-Species Growth and Biofilm Formation

Primary Author Block:
G. Gu1, Y. Xu2, J. Zheng3, S. Bolten4, E. Reed3, Y. Luo5, S. Rideout1, X. Nou5; 1Virginia Tech, Painter, VA, 2Henan Univ. of Technology, Kaifeng, China, 3FDA, College Park, MD, 4Univ. of Maryland, College Park, MD, 5USDA, Beltsville, MD

Abstract Body:
Background: Listeria monocytogenes is one the major foodborne pathogens causing multiple recalls of food products and listeriosis outbreaks in recent years. Our previous studies indicated that the biofilm formation capacity in the dual-species culture of L. monocytogenes and Ralstonia insidiosa, an environmental strain isolated from the contact surface in a produce processing facility, is significantly stronger than that in each mono-culture. However, the mechanism how R. insidiosa promotes the growth and biofilm formation of L. monocytogenes as well as the interaction are still unclear. Methods: Therefore, RNA-Sequencing was applied to analyze the whole transcriptomes of the planktic and biofilm cells among the mono- and dual-species cultures, which were incubated in 50% TSB at 30 oC for 24 h. Results: Over 500 differentially expressed genes were identified, and subsequent analysis indicated that multiple genes which have been reported to be positively correlated with L. monocytogenes biofilm formation, such as a flagellin protein and a glycosyl transferase family protein encoding genes, were up-regulated in the dual-species culture, especially in the cells collected from biofilm. The transcription level of one coding gene of D-Ala-teichoic acid biosynthesis protein, which has been proposed to suppress the biofilm formation of L. monocytogenes, was down-regulated in the biofilm cells of dual-species culture. Additional genes that involved changes in transcriptional regulators, DNA-damage-inducible DNA polymerase, bacterial growth and metabolism were also identified, which indicated R. insidiosa in the dual-species culture might also benefit the growth of L. monocytogenes by supplementing nutritional requirements and other mechanisms. Conclusions: The information derived from this study further contributes to our understanding of L. monocytogenes biofilm formation and survival in the food production environment.
Session Number: 285
Session Type: Poster Talk
Session Title: Biofilms - Loving Relationships?
Session Start Date Time: 6/9/2018 12:15:00 PM
Session End Date Time: 6/9/2018 1:15:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 8706
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Kathryn McBride; 1
Abstract Body:
Abstract Title:
Control of Carbapenem-Resistant Klebsiella Pneumoniae Biofilms Using A Nonionic Surfactant

Primary Author Block:
M. Mazher, A. J. Santiago, R. M. Donlan; CDC, Atlanta, GA

Abstract Body:
Introduction: Handwashing station sink drains are potential reservoirs for carbapenem-resistant Enterobacteriaceae (CRE), including carbapenemase-producing Klebsiella pneumoniae KPC+ (CRKP). CRKP have been shown to colonize sink drain P-traps and form biofilms, and are difficult to eradicate using traditional treatment strategies. The goal of this study was to identify a treatment strategy that could disperse CRKP biofilms without the use of disinfectants or biocides, which may be ineffective against biofilm organisms in these systems. Nonionic surfactants, such as polyoxyethylene-polyoxypropylene block copolymer surfactants (EO/PO BCS) can alter the surface tension at the biofilm/substratum interface and allow removal of biofilm-associated cells. We investigated the ability of P103, an EO/PO BCS to disperse biofilms of K. pneumoniae 1016 KPC+ (pKPC_UVA010) (K. pneumoniae 1016). Methods: For all experiments, K. pneumonia 1016 biofilms were grown in 96-well microtiter plates for 48 h at 37°C. Biofilms were exposed to a range of P103 concentrations (0.25 - 250 mg/L) for 0.5, 1, 3, or 24 h and treated 1, 2, or 3 times. Efficacy was determined by quantifying residual biofilm biomass using the crystal violet assay. Results: A single treatment of P103 was unable to reduce K. pneumoniae 1016 biofilm counts at all concentrations and all time points tested. However, three consecutive 30-min treatments of 0.25 or 0.5 mg/L reduced biofilms (p<0.05) by 20% and 13%, respectively. Three consecutive 1-h treatments of 0.25, 0.5, 1.0, and 2.0 mg/L reduced biofilms (p<0.05) by 24, 33, 21, and 18 %, respectively. P103 treatments greater than 2.0 mg/L were ineffective. Conclusions: P103 surfactant demonstrated dispersal activity against biofilm-associated K. pneumoniae 1016. Multiple treatments at lower use concentrations were significantly more effective. Future investigations will investigate the mechanism for P103 effectiveness against established biofilms and evaluate a treatment strategy incorporating P103 to control CRKP in native polymicrobial biofilms of sink drain P-traps in handwashing sinks of healthcare facilities.
Contamination of combat trauma wounds with environmental residues can lead to bacterial infection of orthopedic fractures, which causes delay and difficulties in patient treatment. The reported infection rate of injuries to U.S. troops in the period of 2003 to 2007 from improvised explosive devices (IED) was reported as 91%, and biofilm formation on orthopedic implants can lead to chronic infection with a rate of 40% in fracture wounds. Once the biofilm has formed, it will become resistant to antibiotics. Therefore, this study focused on designing orthopedic implants that could self-regulate local infection and biofilm formation. Polytetrafluoroethylene (PTFE) and biodegradable chitosan with local antibiotic (vancomycin) elution were deposited onto coupons of stainless steel and titanium alloys which are utilized as implant materials. The study looked at the response of Staphylococcus aureus, which is the most common pathogen associated with orthopedic implant infections. The response of the S. aureus Seattle 1945 (ATCC 25923) strain encoding intracellular GFP was evaluated utilizing crystal violet analysis, ultrasound water bath with viable cell counts and confocal laser scanning microscopy. The release rate of vancomycin from the coupons was also monitored through HPLC analysis of collected leachates from surface modified coupons. In vitro studies of antibacterial properties of the coupons showed that coupons with PTFE did not provide significant advantages against biofilm formation. However, coupons which were coated with chitosan and vancomycin prevented biofilm formation during the in vitro studies. LCSM scanning of the modified surfaces with vancomycin did not indicate the detection of any GFP signal. In addition, no bacterial cells were recovered from the vancomycin treated coupon surfaces. Local drug-release profile of antibiotic doped chitosan showed the concentration of local vancomycin released within the first 48 hours was effective in preventing bacterial attachment onto the coupons. Based on data obtained from these in vitro studies, it is concluded that vancomycin treated coupons were able to successfully prevent biofilm formation and bacterial growth on the modified surfaces.
Abstract Title:
The Effect of Starvation on Bacterial Survivability in Sand and Evolution of Biofilms: A Multi-Scale Study

Primary Author Block:
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Abstract Body:
Background: Formation of biofilms in soil offers a sustainable solution for many geotechnical problems such as soil erosion and contamination. However, little is known regarding how and for how long bacterial biofilms are sustained in soil environments with low nutrient availability. Here, the effect of nutrients’ starvation on biofilm growth and evolution of extracellular polymeric substances (EPS) of Pseudomonas putida in sand columns was investigated. Methods: At the macroscale, static biofilms of P. putida were grown under poor (mineral medium) or rich (mineral medium supplemented with glucose (1 g/L)) nutrients’ conditions for 80 days. Temporally, biofilms were assayed for bacterial growth kinetics (colony forming unit (CFU)) and the quantity of proteins and carbohydrates present in their EPS. At the nanoscale, biological force microscopy was used to quantify the adhesion forces acting between the biofilms and a model surface in saline. To prepare the biological probes, P. putida biofilms were grown on tipless cantilevers for 35 days under both poor and rich nutrient’ conditions. Results: Our macroscale results indicated that the CFU count decreased by two fold 21 days after starvation and remained constant after that, while the fed bacterial CFU count reached a maximum at day 20 and stayed constant after that. Carbohydrates’ concentration of EPS decreased gradually during the 80 days of experiment for both fed and starved biofilms with no statistical significant difference between the two. In comparison, the proteins’ concentration of the starved biofilms increased throughout experiment and that for fed biofilms remained constant throughout. The proteins’ concentration of starved biofilms was 4 fold larger than that of the fed biofilms. Based on our results, we hypothesized that cells under starvation likely synthesize proteins to adhere better to sand. To test our hypothesis, nanoscale experiments of adhesion of starved biofilms for 35 days to a model silicon surface were compared to these of biofilms grown under rich nutrient’ conditions. Our results validated our hypothesis and indicated that the adhesion forces measured with starved biofilms were 4 times those measured with fed biofilms. Conclusions: Our results suggest that, for field studies, administering nutrients once every few months will not disturb biofilm function in soil. Moreover, since the bacterial viability was maintained during the 80 days of experiment, it is expected that biofilm will start to form as soon as the carbon source is supplemented.
Abstract Title:
Ecological Survey of Electrogenic Bacteria Using Microbial Fuel Cells
Primary Author Block:
Z. M. Taylor1, E. Sanchez2, C. Kitts1; 1California Polytechnic State Univ. of San Luis Obispo, San Luis Obispo, CA, 2Allan Hancock Coll., Santa Maria, CA
Abstract Body:
Background: A microbial fuel cell is a bioreactor that utilizes microorganisms to convert chemical energy into electrical energy. Mud is commonly used as both a fuel source and an inoculum especially for benthic fuel cells. Bacterial capacity for oxidizing acetate and depositing electrons directly onto the catalytic surface is influenced by environmental conditions, such as mud and soil type, as well as oxygen and carbon availability. Methods: Fuel cells used carbon felt electrodes, wires and a hacker board outfitted with a capacitor and LED light, all from the MudWatt kit (mudwatt.com). Mud was collected from eight locations around San Luis Obispo County: 4 saline, 2 freshwater, 2 dry soils. Anodes were embedded in soil/mud samples and cathodes placed at the top. Wires embedded through both were hooked to the hacker board. Soils were analyzed to determine pH, %C, %N, %H2O, and conductivity. Voltage and power output were monitored daily for 40 days with a multimeter and the MudWatt app respectively, then T/2 (half the time it takes a cell to reach peak output) was determined. Selected muds were autoclaved or salted to examine the effect of lice organisms and salinity on power output. Acetate was injected into selected low power fuel cells to test for C-limitation. Results: Three soils did not produce significant power over a 60 day incubation. Cal Poly Compost soil (12% C) produced the highest output at 640 mV and 146 uW. When injected with acetate, fuel cell output increased by an average of 146 mV in low performing cells. Peak power output was most highly correlated (0.743) to %C. Mud from saline environments had a high electrical conductivity (average value greater than 10 S) which correlated highly with T/2, but not peak power output. Autoclaved muds produced very low power output, which did not increase over time. Autoclaved cells with marine mud produced an average of 200 mV as did freshwater autoclaved samples with added salt. Conclusions: Electrogenic bacteria are not universally present in soils around San Luis Obispo county as some cells produced insignificant power output over 60 days. Soil carbon content in mud-based fuel cells appears to be the main driver of peak power output. High salinity/conductivity soils can facilitate rapid, low-level voltage generation in mud-based fuel cells. Future efforts will focus on using molecular methods to taxonomically identify the electrogenic bacteria enriched on the anodes of our marine and freshwater mud-based fuel cells.
Simultaneous Bioremediation and Energy Recovery from Abattoir Wastewater Using Copper Anode Microbial Fuel Cell

D. O. Fasheun, E. C. Egwim; Federal Univ. of Technology Minna, Niger, Nigeria

Background: The anode material of a microbial fuel cell (MFC) is very important to its function. Carbon in its different forms, is the most commonly used anode material for electrochemical systems because of its biocompatibility but it suffers a major setback as it has a lower electrical conductivity and higher resistivity than that of metals. Copper is a good conductor but its use in microbial fuel cell (MFC) is limited due to its antimicrobial properties. Abattoir wastewater contains large volumes of organic matter coming from the cow blood, rumen contents and wash water. In many cases, these residual effluents are discharged directly into water bodies thereby putting these ecosystems at risk. In this study, the potential of bioremediation and energy recovery from abattoir wastewater using copper anode MFC was therefore determined.

Methods: Four dual chamber (separated by a rubber latex proton exchange membrane; 3cm x 0.12mm) MFCs were constructed with eight transparent plastic containers (1.2 litre volume each). The anode was Copper coil (28cm x 1.5mm) while the cathode was Aluminium mesh (16cm x 11cm x 1.5mm). Abattoir wastewater served as both the inoculum and organic substrate source for the MFC. A digital multimeter was connected to the MFCs to monitor the open circuit voltage and the voltage across 100ohms, 220ohms, 470ohms and 1000ohms resistors for 8 days at 24hour interval. The corresponding power and power densities were calculated.

Results: The MFC produced an open circuit voltage of 0.895±0.009V. When connected to different resistors, the MFC with the 1000Ω resistor produced the most stable power with a peak voltage and power density of 35mV and 925.8mW/m2 respectively on day 6. The bacterial community analysis of the abattoir wastewater revealed the presence of Bacillus Subtilis, Bacillus megaterium, Staphylococcus aureus, Klebsiella pneumonea, Escherichia coli, and Micrococcus luteus, while only Bacillus Subtilis, and Bacillus megaterium were present in the biofilm formed on the copper anode. After 8 days of operation, the abattoir wastewater became odourless, clearer and electrodes became visible.

Conclusions: This paper demonstrates that despite the antimicrobial properties of copper, exoelectrogens such as Bacillus Subtilis and Bacillus megaterium can form biofilm on the copper anode and facilitate the simultaneous bioremediation and energy recovery from abattoir wastewater.
Abstract Title:
Paenibacillus Sp., A New Plastic Degrading-Bacteria

Primary Author Block:
D. K. R. Bardaji, V. S. Braz, A. F. F. Tonelli, J. A. S. Moretto, E. G. Stehling; Univ.e de São Paulo, Ribeirão Preto, SP, Brazil

Abstract Body:
Background: Plastics are man-made long chain polymeric molecules. The annual production of plastics has doubled over the past 15 years to 245 million tons due to their great physical and chemical properties, thus a large amount of plastic gets accumulated in the environment generating plastic waste ecological problems. The purpose of this study was to isolate bacteria from a waste disposal area with potential to degrade plastic. Methods: These bacteria were isolated in Ribeirão Preto, SP, Brazil using plastic discs as a carbon source and a minimal salt medium (MSM). After genomic DNA extraction, PCR reactions were performed to detect the alkB gene and bacteria that showed this gene were identified and incubated with plastic discs (polyethylene, polyurethane and polyvinyl chloride 5cm discs) and MSM (90mL) for 3 months. After incubation, lose weight measurement analysis, Fourier Transforms Infra-red (FT-IR) analysis, Scanning Electron Microscopy (SEM) analysis and antimicrobial susceptibility tests were performed to evaluate the biodegradation capacity and the resistance profile of the isolates. Results: Five bacteria were isolated, however, only one showed the alkB gene. This bacterium was identified as Paenibacillus sp. using the 16S rDNA gene sequencing. Plastic discs were used normally or chemically treated with tween 80, bleach and ethanol solution and incubated in MSM with the Paenibacillus sp. for 3 months. A significant difference in final weight compared to initial weight was assessed for the 3 types of plastic. Chemical changes were observed by FTIR. The appearance of new functional groups (carboxylic acids (3300-2500 cm-1), esters (1210-1163 cm-1) and ethers (1075-1020 cm-1) and bond scissions were more evident for polyethylene films. SEM visualized physical changes, such as formation of pits and cracks, and bacterial colonization on the plastic surface in all cases. Paenibacillus sp. showed susceptibility to all antibiotics tested except for amikacin. Conclusions: Biodegradation experiments demonstrated the ability of Paenibacillus sp. to modify and colonize plastic. The resistance profile of the isolate showed a low antibiotic resistance providing and additional benefit for its use in bioremediation. Hence this bacterium can be used widely for biodegradation as a promising tool for the elimination of plastic from the environment.
Abstract Title:
Identification of Bacteria from A Soil Community Capable of Growth on A Byproduct of Ethanol Production

Primary Author Block:

Abstract Body:
Background: During commercial ethanol production a liquid syrup byproduct is made in large quantities. This study explored a possible beneficial application of the syrup by using it as a medium for bacterial growth. The long-term goal was to repurpose the resulting bacterial biomass as a protein supplement in the feeds of aquaculture grown animals. Methods: Anaerobic batch reactors were used to enrich for soil bacteria that could use the syrup as the sole nutrient source. Five samples were obtained temporally from eight-day enrichment cultures grown in triplicate to observe shifts in the bacterial community structure. Amplification of the V4 variable region of the 16S ribosomal RNA (rRNA) gene was performed using barcoded primers. The resulting PCR products were sequenced using Illumina MiSeq protocols and analyzed via the program QIIME to observe shifts in the community structure during enrichment. A sample from the last time point was plated onto nutrient-rich agar plates and individual colonies were used to obtain pure culture isolates. Enrichment isolates and other laboratory stock strains were grown in microtiter plates with the syrup substrate and absorbance was monitored for both monocultures and binary combinations. Soil enrichment isolates of interest were subsequently identified at a species level using the full 16S rRNA gene and other biomarkers. Results: The alpha-diversity calculated within each reactor decreased and then increased slightly in the last time point as the enrichments progressed, showing the succession of organisms during growth on the syrup. Community changes revealed enrichment of seven bacterial families at the final time point across the three replicates: Clostridiaceae, Alicyclobacillaceae, Ruminococcaceae, Burkholderiaceae, Bacillaceae, Veillonellaceae, and Enterobacteriaceae (in order of decreasing total percentage present). Bacillaceae were recovered at the highest frequency of pure culture isolates. Results from microtiter growth plates indicated Bacillus species, commonly used as probiotics in aquaculture, have the highest growth rate and yield of all the monocultures examined. Binary combinations of the isolates yielded no significant synergism between organisms, suggesting competition for nutrients instead of beneficial cell-cell interactions regarding metabolite conversion. Conclusion: Bacteria from pure stock cultures and from an enriched soil sample utilized the syrup as a growth substrate under facultative conditions with Bacillus species having the highest yields.
Session Number: 325
Session Type: Rapid Fire
Session Title: Bugs that Fuel and Clean Up
Session Start Date Time: 6/9/2018 3:00:00 PM
Session End Date Time: 6/9/2018 3:45:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 8774
Poster Board Number:

Abstract Title:
Shining Light on Anoxygenic Phototrophic Bacteria: A Syntrophy of Teamwork for Hydrocarbon Degradation
Primary Author Block:
T. C. Reid1, I. G. Droppo2, S. Chaganti1, C. G. Weisener1; 1Univ. of Windsor, Windsor, ON, Canada, 2Environment and Climate Change Canada, Burlington, ON, Canada

Abstract Body:
Background: Microbial populations in both aquatic and terrestrial environments deal with a constant flux of nutrients and xenobiotic substances both naturally and resulting from anthropogenic influences. Advancements in genetic sequencing techniques is providing great insight is into how microbes are dealing with such changes, and how microbes have and continue to alter global biogeochemical cycling. Fingerprinting these types of compromised environments has become a dominant topic of interest within the scientific community, though what often remains unstudied are natural/baseline environments, providing crucial “reference” parameters from which to compare these contaminant sites. In terms of bitumen mine reclamation, the case for understanding baseline environments is perhaps most pertinent in End-Pit Lake (EPL) reclamation research. These EPLs are a proposed reclamation strategy once mining has ceased, where extraction wastes (water, clays, sands, residual bitumen etc.) are pumped into the leftover mine pits. However, to date, little is known about the long-term fate of these proposed EPLs, and even less is known about the functional capabilities of the indigenous microbial populations, particularly their biodegradation potential. Methods: Accessed via helicopter, sediment cores were taken from several sample sites transecting hydrocarbon-rich water reservoirs in Alberta, Canada. RNA was extracted from preserved sediment aliquots, check for QC, and sequenced on the Illumina HiSeq 2000 at Genome Quebec. Characterization of energy and xenobiotic degradation processes was performed following processing with Metatrans Pipeline and DESeq2.
Results: Differential gene expression analysis revealed significant variation between sample sites, driven by oxygen availability at the sediment-water interface. A unique syntrophy of metabolism was observed between phototrophic organisms, sulfate reducers and methanogens. Xenobiotic degradation transcripts indicate ongoing biodegradation because of natural hydrocarbon presence. Conclusions: Observed trends in gene expression indicate a cooperative metabolism between microbial species, reliant on both oxygencic and anoxygenic photosynthesis. The complexity of the syntrophic interactions observed between microbial species provides context as to how these microbes can be so metabolically efficient at degrading compounds in a theoretically unfavorable thermodynamic environment.
Abstract Title:
Role of the Megaplasmid Pswit02 in Dioxin & Dibenzofuran Degradation by Sphingomonas Wittichii Rw1
Primary Author Block:
S. S. Eleya, G. Zylstra; Rutgers Univ., New Brunswick, NJ
Abstract Body:
Sphingomonas wittichii RW1 metabolizes dioxin and dibenzofuran as sole carbon and energy sources. Sphingomonas wittichii RW1 is considered a model organism that facilitates the study of the molecular and biochemical mechanisms of dioxin and other environmental toxicants metabolism. The complete genome sequence of RW1 reveals that this strain, in addition to a main chromosome contains two circular megaplasmids pSWIT01 and pSWIT02. A number of studies have suggested that pSWIT02 carries genes that are involved in dioxin and dibenzofuran degradation. However, recent work in our laboratory has shown that the ring cleavage dioxygenase (second enzymatic step) and hydrolase (third enzymatic step) of the dioxin catabolic pathway are encoded by the chromosome. It is our hypothesis that the megaplasmid pSWIT02 only encodes the first enzymatic step of the dioxin pathway and we aim in this study to better clarify the role of pSWIT02 in Sphingomonas wittichii RW1. We constructed a new plasmid (pSEZ_RW1) containing the origin of replication oriR and associated partition (par) genes of pSWIT02 along with genes for tetracycline resistance. This plasmid was mated into RW1 with the goal of forcing out the megaplasmid pSWIT02 since the shared oriR and par would result in incompatible plasmids. The dual plasmid construct was subcultured multiple times in LB liquid medium and colonies were examined for the presence of pSWIT02 by PCR. This eventually resulted in a pSWIT02 cured version of RW1. The pSEZ_RW1 plasmid is slightly unstable and loss of this plasmid was then obtained by selecting for colonies lacking tetracycline resistance. Unsurprisingly, the pSWIT02 cured strain did not grow on either dioxin or dibenzofuran. We used PCR to clone the dxnA1A2, fdx3, and redA2 genes encoding a multicomponent angular dioxygenase from three different locations in pSWIT02 into the low copy number vector pRK415 so that expression of the genes is from the lac promoter. Moving this plasmid into the cured RW1 restored growth on dioxin and dibenzofuran. Growth curves on minimal medium supplemented with either compound as the sole carbon source showed that the rate and extent of growth was almost the same as the wild type strain. Based on these experiments we conclude that the only pSWIT02 genes involved in the degradation of dioxin and dibenzofuran are the ones encoding the initial angular dioxygenase. This explains why very few dioxin degrading organisms are known, that a combination of plasmid and chromosome encoded genes are necessary for growth on this recalcitrant compound.
Abstract Title:
Tailoring Pseudomonas Putida for Valorizing Toxic Biomass-Derived Compounds

Primary Author Block:

Abstract Body:
Thermochemical (TC) biomass conversion processes such as pyrolysis are promising technologies for the sustainable production of fuels and chemicals from lignocellulose. However, these processes generate a considerable amount of organic-rich, heterogenous, highly toxic wastewater streams, which are challenging to convert via standard wastewater treatment approaches without a priori detoxification strategies, as well as representing a process cost for the TC biorefinery. To adapt the biological funneling concept for valorizing waste carbon in a TC process, we first comprehensively characterized a range TC wastewater streams from pilot-scale operation, which led to identification and quantification of around 200 compounds including aldehydes, ketones, acids, aromatics, and sugars at near-complete mass closure. Based on the compositional analysis, we employed the robust and metabolically versatile microbe, Pseudomonas putida, as a biocatalyst for valorization of these streams. However, the extreme toxicity of these streams hampers P. putida growth and carbon utilization. Analysis of the chemical toxicity of the TC streams reveals that aldehydes are by far the most inhibitory compounds in these streams to P. putida. Multi-omics and biochemical analyses of P. putida grown in a TC wastewater streams suggest that protein damage is one of the key components of toxicity. To overcome acute protein damage under TC wastewater chemical stress, we engineered the native protein quality control machineries in a genome-reduced P. putida strain (EM42). The engineered strain improves the tolerance towards multiple TC wastewater samples in some cases up to 200-fold. The engineered strain can utilize TC wastewater carbon at an industrially process-relevant concentration without a priori detoxification as its sole source of carbon and energy. As an initial proof-of-concept, we demonstrate that the engineered strain can produce polyhydroxyalkanoates as well. When coupled to other metabolic engineering advances, such as expanded substrate utilization, this study enables new avenues of biological conversion of biomass-derived waste streams via an aerobic, engineered P. putida monoculture.
Abstract Title: Microbial Degradation of Art-Waste Solvents
Primary Author Block: C. Oberg, G. McKay, M. Culumber, E. Walker; Weber State Univ., Ogden, UT
Abstract Body:
Paint and solvents used in acrylic and oil painting generates waste that is resistant to chemical breakdown, requires expensive disposal fees, and causes health hazards during storage. Painting byproduct storage containers were found to have bacteria growing in them that could be metabolizing paint waste. Microbial degradation of three paint solvents, linseed oil, bestine, and turpenoid, by bacteria isolated from paint waste containers was investigated. In addition, bacterial strains previously isolated from jet fuel-contaminated soil were also tested for their ability to degrade these three solvents. All bacterial isolates were propagated in M9 minimal media broth containing each solvent with the majority forming biofilms at the solvent/broth interface after three weeks of incubation at 22oC. Eight of 16 isolates have been identified by 16S rRNA sequencing with taxonomic analysis of remaining isolates underway. Identified isolates from paint waste containers include Pseudomonas zhaodongensis, Planococcus citreus, and Planococcus rifletoensis. Gas chromatography mass spectrometry (GC/MS) was used to measure microbial degradation of two solvents. GC/MS results indicate six bacterial isolates degrade both bestine and oleic acid, a selected component of turpenoid, as a number of new peaks (breakdown products) were detected and the initial solvent peak areas decreased over time. Results show bacterial strains isolated from the paint waste and from jet fuel-contaminated soil have the ability to degrade individual paint waste solvents. Optimizing growth conditions (pH, oxygen, and temperature) indicates modest changes in container handling can maximize solvent biodegradation. Once the most efficient bacterial strains and their optimum growth parameters are identified, they could be inoculated into waste containers to degrade paint waste, reducing disposal fees and health risks.
Session Number: 325
Session Type: Rapid Fire
Session Title: Bugs that Fuel and Clean Up
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Session Primary Track: Applied and Environmental Science
Abstract Control Number: 9131
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Ann M. Stevens; Virginia Tech, Blacksburg, VA
Abstract Body:
Abstract Title:
Isolation and Dev. of Bacillus Megaterium Sr7 For Biofuel Production and Recovery Under Supercritical Co2

Primary Author Block:
A. J. E. Freedman1, J. Boock1, G. Tompsett2, M. T. Timko2, K. L. J. Prather1, J. R. Thompson1;
1Massachusetts Inst. of Technology, Cambridge, MA, 2Worcester Polytechnic Inst., Worcester, MA

Abstract Body:
Supercritical carbon dioxide (scCO2) is an attractive substitute for conventional organic solvents due to its unique transport and thermodynamic properties, its renewability and labile nature, and its high solubility for compounds such as alcohols, ketones and aldehydes. However, biological systems that use scCO2 are mainly limited to in vitro processes due to its strong inhibition of cell viability and growth. To solve this problem, we used a bioprospecting approach to isolate a microbial strain capable of growth under scCO2. Enrichment culture and serial passaging of deep subsurface fluids from the McElmo Dome scCO2 reservoir in biphasic aqueous media with scCO2 headspace enabled the isolation of spore-forming Bacillus megaterium SR7. Sequencing and analysis of the complete 5.51 Mbp genome and physiological characterization revealed the capacity for facultative anaerobic metabolism, including fermentative growth on a diverse range of organic substrates. Supplementation of growth media with L-alanine for induction of spore germination significantly improved growth frequencies and biomass accumulation under scCO2 headspace. Detection of endogenous fermentative compounds in cultures grown under scCO2 represents the first observation of bioproduct generation and accumulation under this condition. To leverage SR7's biocompatibility with scCO2 we sought to genetically modify the strain to generate products that would partition into scCO2. Transformation of SR7 was achieved using a protoplast-based method, permitting identification of promoters for inducible heterologous protein expression in both aerobic and anaerobic conditions. We engineered SR7 to produce isobutanol by a two-enzyme (2-ketoisovalerate decarboxylase (KivD) and alcohol dehydrogenase (Adh)) pathway. A library of Adh proteins was screened to identify enzymes that rapidly convert the isobutyraldehyde intermediate since this compound is expected to highly partition into the scCO2 phase. Combining our recombinant biofuel strain with scCO2 culturing, isobutanol production was observed with co-production of isopentanol, representing the first recombinant bioproducts generated from bacteria grown under scCO2. For cultures that showed high metabolic activity under scCO2, we found almost 50% conversion of the α-ketoacid substrate to biofuel product. Culturing, development and metabolic engineering of B. megaterium strain SR7 represent initial efforts towards enabling exploitation of scCO2 as a sustainable solvent for in vivo bioprocessing.
Abstract Title: Turning Plastic Waste Into Added Value Materials
Primary Author Block: I. Radecka, B. Johnston, D. Hill, M. Kowalczyk; Univ. of Wolverhampton, Wolverhampton, United Kingdom
Abstract Body: Mountains of plastic wastes are buried in landfill sites, also millions of tonnes of plastic waste leaks into our oceans each year. This continues to pose a growing challenge for authorities around the world [1,2]. Naturally-occurring bacterial polymers have an enormous potential as they can be produced from renewable resources under well controlled conditions. Polyhydroxyalkanoates (PHAs) are a group of biocompatible, environmentally neutral, biodegradable plastics that can be produced by certain bacteria [1]. One of the factors limiting the mass usage of PHAs is the high cost of the carbon sources required by microbial cells and the expensive processing requirements to extract and develop stable PHA structures in comparison to the wide range of petrochemical plastics currently in use [2]. Attempts are therefore being made to find new ways in which to increase the rate and efficiency of microbial synthesis of bioplastics. The objective of this work was to develop a viable biotechnological process in which a waste plastic can be turned by bacteria into added value materials. This study introduced the novel use of oxidized polyethylene wax (O-PEW) and non-oxidized polyethylene wax (N-PEW) substrates carbon sources for bacteria [3]. These waxes were then fed to the bacteria to make PHAs. The bacterial strain of Cupriavidus necator H16 was selected for the study as it been reported to utilize a wide range of carbon source including fatty acids for PHA production. C. necator H16 was grown for 48 hours in nitrogen rich or nitrogen-limited media that were supplemented with O-PEW or N-PEW [5]. Under those conditions the accumulation of PHAs varied from 20% to 40% (wt / wt) of dry biomass in both media. All bacterial polymers produced were analysed using NMR, GPC and they were further evaluated with ESI-MS/MS. Analysis revealed that the PHAs obtained contained 3-hydroxybutyrate and up to 3 mol % of 3-hydroxyvalerate as well as 3-hydroxyhexanoate co-monomeric units [3, 4]. It can be concluded, that both waxes could be a promising carbon source for PHA production. Obtained data provide a strong ‘proof of concept’ that the addition of PE waxes to the microbial growth medium can have an influence on the structure of bacterial polyesters made and their chemical properties. This study demonstrates that PHA producing bacteria can contribute to the solution of the problem of disposal of manufactured plastics.
Abstract Title:
Presence and Distribution of Carbapenem-Resistant Gram-Negative Bacilli in One Municipal Wastewater Treatment Plants of Colombia

Primary Author Block:
E. A. Rodriguez1, A. Aristizábal2, S. Morales2, L. Arias3, J. N. Jimenez2; 1Applied & Basic Microbiol. Res. Group MICROBA and Molecular Microbiol. Group. Univ. of Antioquia, Medellin, Colombia, 2Applied & Basic Microbiol. Res. Group MICROBA. Univ. of Antioquia, Medellin, Colombia, 3Microbial Bioprocesses group BIOMICRO. Univ. of Antioquia, Medellin, Colombia

Abstract Body:
Background: The wastewater treatment plants (WWTP) are considered one important reservoir of bacterial resistance. Carbapenem-resistant Gram-negative Bacilli have been described in different WWTP in the world, however, few studies describe the distribution of these microorganisms in different points of WWTP. The objective of this work is to determine the presence of Carbapenem-resistant Gram-negative Bacilli along WWTP in Colombia.

Methods: A cross-sectional study was conducted in one WWTP in Medellin. Once a month from January to July 2017, water samples were taken from 4 points along WWTP, influent, aerated tank, recycled sludge and effluent. Isolation of bacteria was performed on chromID® CARBA medium. Bacteria were identified by 16S rRNA and susceptibility testing was performed using the VITEK®-2. Molecular analyzes included PCR for detection of blaKPC, blaNDM and blaOXA-48 genes. Results: A total of 360 Gram-negative bacilli were isolated on ChromID carba plates (90 isolates for point). The carbapenem-resistant bacteria detected were Aeromonas spp (n = 58, 41%), followed by Enterobacter spp (n = 38, 27%), Raoultella sp. (n = 12, 8%), Klebsiella spp (n = 11, 8%), Citrobacter freundii (n = 8, 6%), Pantoea sp. (n = 7, 5%), Kluyvera spp. (n = 4, 3%), Escherichia coli. (n = 3, 2%) and Pseudomonas sp. (n = 1, 1%). High minimum inhibitory concentration (MIC) values of carbapenem were observed in these bacteria and their resistance pattern showed resistances for two or more tested antibiotics. The carbapenemase KPC was detected in 142 isolates resistant to carbapenem and was more frequent in isolates of influent (n = 44, 49%) and effluent (n = 41, 44%) and less frequently in the recycled sludge (33%, n = 30) and the aerated tank (31%, n = 28). No other carbapenemases were detected. In general, the bacteria harboring KPC were detected in different sampling sites, but Aeromonas spp. were more frequently detected in recycled sludge.

Conclusions: This study demonstrates the high prevalence carbapenem-resistant Gram-negative bacilli in different sampling sites of one WWTP, this shows the risk of dissemination of multidrug-resistant genes between environmental and opportunistic pathogens.
Can Elevated Salinity Trigger the Viable But Nonculturable State in the Marine Pathogen Vibrio Vulnificus?

Primary Author Block:
B. McHenry, G. Barbarite, P. J. McCarthy; FAU Harbor Branch Oceanographic Inst., Fort Pierce, FL

Abstract Body:
Vibrio bacteria are responsible for 80,000 illnesses in the United States every year, the majority of which occur in Florida. One species, Vibrio vulnificus, can cause potentially fatal wound infections in a select group of the population. These bacteria are found worldwide in estuarine environments but are never recovered on beaches or in oceanic waters. A field study was conducted to test the abundance of V. vulnificus along a salinity gradient from inshore to offshore. This research showed that these pathogens exhibit a strong negative correlation with salinity, rarely being found in areas approaching 35 ppt. To further investigate this trend, a lab study was conducted to monitor the culturability of V. vulnificus cells exposed to an elevated, sub-optimal salinity. Cells were grown in Heart Infusion broth overnight and washed twice to remove excess nutrients. Stocks were then inoculated into a control microcosm (11 ppt salinity) and a treatment microcosm (35 ppt salinity) at a density of 106 cells/mL. The number of culturable V. vulnificus was determined for both the control and treatment microcosms by plating onto CHROMagar Vibrio followed by overnight incubation at 37°C. The control microcosm maintained an average of 1.57 x 106 culturable cells/mL throughout the trial, while the treatment culture began to decline by day 1; no cells could be recovered at elevated salinity after day 7. These data suggests that elevated, oceanic salinity can significantly reduce the culturability of Vibrio vulnificus in as little as 7 days. These results may also imply the induction of the Viable But Nonculturable (VBNC) state of V. vulnificus in response to sub-optimal salinity. The VBNC state is an environmental stress response which has been studied most frequently for V. vulnificus at sub-optimal temperatures. While V. vulnificus has been shown to enter the VBNC state in response to decreased temperatures, this is the first study to indicate that this pathogen may be able to elicit the same response when exposed to elevated salinity. Understanding the tolerance range of this bacterium as well as its responses to various environmental stressors is crucial to informing the public and health care providers about this potentially fatal human pathogen.
Flooding is Associated with Increase in Relative Abundance of Aspergillus and Penicillium Spp. in Built Environment: Implications to Human Health

Primary Author Block:
P. Gummadidala1, C. Mitra1, M. Omebeyinje1, G. Scott1, N. Patel2, F. Valentine2, V. Thomas2, A. Chanda1; 1Univ. of South Carolina, Columbia, SC, 2All Solutions, Inc, Livingston, NJ

Abstract Body:
United States experiences approximately 5 million cases of respiratory problems that are largely correlated with exposures to Aspergilli and Penicillium in built environments. This leads to an estimated healthcare cost of approximately $3.5 billion. Since Americans spend 90% or more of their time indoors, mold related health costs are likely to increase with the rise in weather events such as hurricanes and tidal floods. Current evidence suggests that Aspergillus and Penicillium are largely associated with water-damaged buildings. However, it is not understood whether flooding caused by such extreme weather activities determine the relative abundance of these mold genera in built environment. To address this knowledge gap we conducted a survey of flooded and non-flooded buildings (both residential and non-residential; n=6 per group) that were impacted by two major weather events in South Carolina: the ‘100 year flood’ and hurricane Matthew. Air sampling in these moldy buildings were performed using ‘settle plate’ technique in which the number of mold colonies growing on potato dextrose agar plates upon 30 min of air exposure were analyzed. Colonies that grew on the plate after 5-7 days of incubation at 30oC were identified colony-by-colony using internal spacer sequence 1 (ITS1) analysis coupled with phylogenetic identification of the species. Our results demonstrated significant increase (> 2 fold) in relative abundance of Penicillia and/or Aspergilli in homes where mold problems were flood-associated, as compared to non-flooded homes. Finally, we investigated indoor air samples of buildings (n=6) in New Jersey, which experienced mold problems five years after flooding post hurricane Sandy. We observed a 90% abundance of Penicillium spp. in all these homes, suggesting that the dominance of flood-associated rise of these genera in built environment is long-lasting. Finally, a retrospective review of existing literature on the most abundant Aspergillus/Penicillium spp. identified in our survey include A. flavus, A. fumigatus and P. rubens which are well known pathogens and associated with fatal respiratory and neurological illnesses in immunocomprised patients, children and the elderly. Our findings support our proposed model that contaminants in floodwater may provide new nutrient source for selective overgrowth of pathogenic molds from genera Aspergillus and Penicillium, their sporulation and the emission of their VOCs, which can collectively deteriorate the indoor air quality and detrimentally impact human health.
Abstract Title:
Chemical and Microbial Analysis of Recreational Freshwater in and Around Blue Marsh Reservoir, Berks County, Pa

Primary Author Block:
Z. T. Weagly, J. M. Felker, T. H. Mysliwiec; The Pennsylvania State Univ., Reading, PA

Abstract Body:
Human use areas along selected waterways expose individuals to bacterial populations with unique biochemical characteristics and potential pathogenic properties. Initial visual observations from three sites (upstream, downstream, and reservoir) along a heavy human use waterway in central Berks County, Pennsylvania indicated that different regions of the same creek comprise different levels of algal growth, invertebrates, and plant life. The working hypothesis for this study stated that the downstream site would have a vastly different microbial population from the upstream site. Testing was performed on these areas to determine differences in the water chemistry and the microbial populations. Chemical testing included; pH, temperature, NO3–, PO43–, and BOD. Dramatic variations in pH were observed and key differences in nitrate levels were found for all sites tested. Microbial tests included determination of preferential food sources, relative abundance, and overall colony forming units (CFU’s). CFU’s were determined using three independent plate counts looking for Escherichia coli, heterotrophs, and Enterococcus spp. Results from the CFU’s testing at each location varied seasonally, both amongst the different and within each site. Initially the upstream location contained higher counts of CFU’s for all species tested, however, the collection site within the reservoir, where human use was highest, showed higher counts after the warm season. As expected, human use areas were shown to have higher counts of E. coli and Enterococcus spp. BioLog Ecoplates were inoculated to examine variation in biochemical utilization by the microbial populations at all sites and to determine the preferred carbon sources of local microbial populations. Ecoplate results identified the presence and apparent preference for synthetic carbon sources as primary metabolites. Analysis identified Tween 40 and Tween 80 as two preferred carbon sources. Both have been identified as chemicals which can limit the effectiveness of certain antibiotics used to combat bacterial infections. The presence of this particular carbon source is likely due to common agricultural practices in the area. 16S ribosomal sequencing of select samples is currently underway.
Abstract Title:
High-Resolution Spatial-Temporal Dynamics of Escherichia coli in An Urban Stream System
Primary Author Block:
Abstract Body:
In urban landscapes, aging sewage infrastructure, leaky sewer lines, failing septic systems, and increasing amounts of impervious surfaces can impair water quality and affect public health via introduction of enteric pathogens into surface waters. Detecting these impacts requires active surveillance. In this study, a network of stream sites around Athens, Georgia (USA), an urban area home to ~100,000 people served by sewers (>100 years old), septic systems, and bounded by agricultural land, were sampled weekly for Escherichia coli, a fecal indicator bacteria, over a 15-month period (October 2016 to December 2017). Over this sampling campaign, 228 stream samples were collected at 9 stations. For all samples collected, E. coli concentrations were determined using EPA Method 1603. Over the 15-mo period, E. coli counts averaged 1,055 CFU 100 ml-1 and ranged from a low site average of 444 CFU 100 ml-1 (n = 25) in residential areas to a high site average of 1,747 CFU 100 ml-1 (n = 28) on the University of Georgia campus. Additionally, two sites immediately downstream of a hospital sewer line had average E. coli levels of >1,300 CFU 100 ml-1 (n = 28,39) as well as the highest single sample value of >12,000 CFU 100 ml-1; although numbers were high year-round, the highest counts were observed during spring and early summer suggesting consistent inputs of fecal material. Additionally, a synoptic sampling campaign was conducted across 40 stations to assess site variability within a 4 h time window (November 2017). In this collection, E. coli averaged 2,046 CFU 100ml-1 (ranging from 270 to 9,550 CFU 100 ml-1). Synoptic samples were also analyzed for extended spectrum beta lactamase (ESBL) producing enterobacteriaceae using chromogenic agar followed by PCR. E. coli isolates PCR-positive for both bla CTX-M (cefotaximase) and blaTEM (penicillinase) genes were detected in an urban stream segment (n=2) and in wastewater influent (n=1). A blaCMY (carbapenemase) positive E. coli isolate was also detected in an adjacent stream site. These preliminary data point to possible antibiotic resistance activity in urban waters.
Session Number: 349
Session Type: Rapid Fire
Session Title: Pathogens in the Drink
Session Start Date Time: 6/9/2018 4:30:00 PM
Session End Date Time: 6/9/2018 5:00:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 9132
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Erin K. Lipp; Univ. of Georgia, Athens, GA
Abstract Body:
Session Number: 381
Session Type: Poster Talk
Session Number: 381
Session Type: Poster Talk
Session Title: Microbes and New Ideas - Stretching the Boundaries
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/7/2018 1:35:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 8707
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
LIYOU WU; 1
Abstract Body:
Abstract Title:
Improved Microbial Source Tracking with Digital Droplet PCR
Primary Author Block:
J. Nshimyimana1, C. Cruz1, S. Wuertz1, J. R. Thompson2; 1Nanyang Technological Univ., Singapore, Singapore, 2Singapore-MIT Alliance for Res. and Technology, Singapore, Singapore
Abstract Body:
We addressed whether digital droplet PCR (ddPCR) could improve sensitivity and specificity of human-associated Bacteroidales genetic markers and their quantification in environmental and fecal composite samples. BacHum and B. theta, previously validated for microbial source tracking in Singapore and Southeast Asia (Nshimyimana et al, 2017), were tested in 180 samples and quantified by qPCR and ddPCR (n = 35 human stool, n = 70 domestic and wild animal feces, n = 20 sewage, n = 20 environmental and n = 35 composite samples). Quantification of BacHum by ddPCR increased specificity (from 0.63 to 0.88) and accuracy (from 0.80 to 0.93) relative to qPCR, while the B. theta marker performed similarly on both platforms (specificity = 0.98 for qPCR and ddPCR). DdPCR and qPCR quantification of environmental and fecal composite samples were highly correlated (R > 0.87, p<0.0001, n = 110) where concentrations measured by ddPCR were consistently lower than those measured by qPCR, by a factor of 2.6 ± 2.8 for B. theta and by a factor of 11.8 ±7.8 for BacHum. When qPCR standard curves were calibrated based on ddPCR-based measurement of standards, closer agreement between qPCR and ddPCR measurements was obtained with near perfect agreement for B. theta and 2.3-fold higher qPCR values for BacHum. Thus, our work suggests ddPCR improves quantification of samples with low target concentrations by removing systematic errors associated with dilution-based qPCR standard curves. We conclude that ddPCR is a suitable tool for microbial source tracking; however, other factors such as cost-effectiveness should be considered.
Mountains of plastic wastes are buried in landfill sites, also millions of tonnes of plastic waste leaks into our oceans each year. This continues to pose a growing challenge for authorities around the world [1,2]. Naturally-occurring bacterial polymers have an enormous potential as they can be produced from renewable resources under well controlled conditions. Polyhydroxyalkanoates (PHAs) are a group of biocompatible, environmentally neutral, biodegradable plastics that can be produced by certain bacteria [1]. One of the factors limiting the mass usage of PHAs is the high cost of the carbon sources required by microbial cells and the expensive processing requirements to extract and develop stable PHA structures in comparison to the wide range of petrochemical plastics currently in use [2]. Attempts are therefore being made to find new ways in which to increase the rate and efficiency of microbial synthesis of bioplastics. The objective of this work was to develop a viable biotechnological process in which a waste plastic can be turned by bacteria into added value materials. This study introduced the novel use of oxidized polyethylene wax (O-PEW) and non-oxidized polyethylene wax (N-PEW) substrates carbon sources for bacteria [3]. These waxes were then fed to the bacteria to make PHAs. The bacterial strain of Cupriavidus necator H16 was selected for the study as it been reported to utilize a wide range of carbon source including fatty acids for PHA production. C. necator H16 was grown for 48 hours in nitrogen rich or nitrogen-limited media that were supplemented with O-PEW or N-PEW [5]. Under those conditions the accumulation of PHAs varied from 20% to 40 % (wt / wt) of dry biomass in both media. All bacterial polymers produced were analysed using NMR, GPC and they were further evaluated with ESI-MS/MS. Analysis revealed that the PHAs obtained contained 3-hydroxybutyrate and up to 3 mol % of 3-hydroxyvalerate as well as 3-hydroxyhexanoate co-monomeric units [3, 4]. It can be concluded, that both waxes could be a promising carbon source for PHA production. Obtained data provide a strong ‘proof of concept’ that the addition of PE waxes to the microbial growth medium can have an influence on the structure of bacterial polyesters made and their chemical properties. This study demonstrates that PHA producing bacteria can contribute to the solution of the problem of disposal of manufactured plastics.
Direct Detection & Quantification of Microbial Contamination Within Home and Personal Care Products

Primary Author Block:
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Abstract Body:
Background: Home and personal care product (HPCP) industries use antimicrobial preservatives to prevent bacterial growth. HPCP formulations consist of proteins and varied carbon sources that facilitate microorganism growth. Early detection during manufacture is thus key to prevent consumer exposure to objectionable organisms such as intrinsically preservative-tolerant Pseudomonas aeruginosa and Burkholderia cepacia complex bacteria. We aimed to detect bacterial contamination directly from HPCPs and explore: (i) if intact metagenomic DNA can be extracted from HPCPs; (ii) the metataxonomic bacterial diversity associated contamination in comparison to routine cultivation-based monitoring; (iii) optimal extraction methods for detecting key contaminant species in a range of HPCPs. Methods: 14 contaminated industrial HPCPs of varied ages were subjected to total DNA extraction using an automated, kit-based method. Bacterial diversity was examined by 16S rRNA gene pyrosequencing and P. aeruginosa specifically detected by oprL and phzS gene-specific PCRs. Optimisation of the DNA extraction and PCR was evaluated across 3 types of HPCP spiked with B. cepacia, P. aeruginosa and Staphylococcus aureus. Organism specific quantitative real-time (B. cepacia rpoD; P. aeruginosa gyrB) and nested PCRs (S. aureus rRNA intergenic spacer region V-VI) were used to determine the detection limits of the cultivation independent procedures. Results: Metagenomic DNA was extracted and amplified using a 16S rRNA gene PCR from 12/14 contamination incident samples; bacterial diversity analysis showed that Pseudomonas was the dominant organism in 7/10 products subject to pyrosequencing. Correlation of culture and culture-independent methods was also observed for samples containing P. aeruginosa and Enterobacter sp. DNA was extracted from all artificially contaminated HPCPs, but were not amplifiable in the case of lotion products. qPCR detected ≥ 103 CFU/ml B. cepacia and P. aeruginosa, while nested PCR detected ≥ 103 CFU/ml S. aureus. Conclusions: Taxonomic profiles were successfully defined using DNA extracted from HPCPs and showed that one contaminant species predominates. Molecular detection methods can be used to detect specific contaminant organisms in a range of HPCPs.
Abstract Title:
The Impact of Storms on Legionella pneumophila Concentrations in Cooling Tower Water

Primary Author Block:
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Abstract Body:
Background: Legionellosis, or Legionnaire’s Disease (LD), is a pneumonia caused by Legionella bacteria that thrive both in man-made water distribution systems and natural surface waters including lakes, streams, and wet soil. LD is typically contracted by inhaling Legionella Disease bacteria (LDB), most often in aerosolized mists that contain the bacteria. Cooling towers have been found to be a source of Legionella in numerous LD outbreaks through aerosolization. Management of cooling towers can be a factor as the presence of stagnant water, lack of maintenance, and/or environmental conditions can cause buildup of Legionella pneumophila, the primary cause of LD. The US Occupational Safety and Health Administration (OSHA) has specific guidelines when LDB concentrations get to 10^6-10^7 cells/L in cooling tower water systems to prevent exposure.

Methods: At the US Department of Energy’s Savannah River Site (SRS) in Aiken, SC, cooling tower water is routinely monitored for L. pneumophila concentrations (serogroups 1, 2, 4, and 6) on a monthly or quarterly basis using a Direct Fluorescent Antibody (DFA) technique. Historically, the 24 operating SRS cooling towers have had varying concentrations of Legionella in all seasons of the year with patterns that are unpredictable. The cooling towers are of varying age, water treatment system, construction, size, water supply, and geographical distribution over the 320 square miles of SRS. A stoplight system based on cooling tower water L. pneumophila concentrations has been developed to help operators control microbial growth.

Results: Environmental conditions can impact L. pneumophila control in cooling towers as observed with extreme 2017 summer weather in data presented here. Cooling tower 785-A/2A concentrations went from averaging 10^5-10^6 cells/L to 10^7-10^8 cells/L after Hurricane Irma and associated extreme weather (Figure 1). Despite automated biocide addition, these increases were likely due to impact of windblown debris into the cooling tower and basin with excessive rain.

Conclusions: A clean out of the cooling tower basin and repeated biocide applications were required to bring L. pneumophila down to 10^5-10^6 cells/L, the safe or “green” level, in this cooling tower water. Twenty-four other SRS towers were not exposed to as much debris as 785-A/2A and did not demonstrate the L. pneumophila increase despite the extra precipitation and varied from not detect (ND) range up to 10^6 cells/L as measured by DFA.
Abstract Title:
Expressing Bacterial Luminescence Genes in Human Cells to Develop Ready-To-Use Drug Screening Tools Suitable for Microgravitational Use Onboard the Intl. Space Station

Primary Author Block:
T. Xu1, S. Ripp1, G. Sayler2, D. Close2; 1The Univ. of Tennessee, Knoxville, Knoxville, TN, 2490 BioTech, Knoxville, TN

Abstract Body:
Human cell culture in microgravity promotes the natural formation of three-dimensional structures without necessitating exposure to exogenous scaffold materials. This endows cells with a more natural physiology and enhances drug discovery by enabling enhanced prognostication of compound efficacy. Unfortunately, the logistical costs of delivering the materials needed for firefly luciferase-based compound evaluation in low Earth orbit have obviated the use of microgravitational research platforms, such as the International Space Station, for this purpose. We are investigating the adaption of the bacterial luciferase gene cassette to serve as a reporter for human cell metabolic impact testing to overcome the logistical hurdles of space-based drug discovery. The bacterial luciferase gene cassette from Photobacterius luminescens was modified for expression in human cells and co-expressed with a supporting oxidoreductase to enable continuous bioluminescent output concordant with the host’s metabolic activity level. A variety of cryogenic preservation and reanimation approaches were evaluated to develop a method whereby cells could be pre-packaged on Earth, stored at -80 °C, then thawed and injected into pre-prepared, drug compound-containing multi-well plates in-flight and used to continuously monitor metabolic activity. This method was tested against five therapeutic compounds with known metabolic impacts. Using the method developed in this work, compound-induced metabolic impacts can be tracked continuously for up to 24 hours. The observed timing of compound metabolic impacts remained consistent (p ≤ 0.08) and ANOVA of signal strengths from replicate assays ranged between 0.49 and 0.77 for all tested chemicals, demonstrating a high level of consistency. Our findings indicate that the use of continuous bioluminescence as a reporter tool produces the necessary detection characteristics to enable metabolic activity/toxicity testing in low Earth orbit. The self-contained nature of the system reduces the number of samples and reagents required and lowers testing costs by limiting the weight and space burdens imposed during delivery to the International Space Station. This system has been cleared for in-flight testing and is scheduled for delivery to the space station for validation on the SpaceX-14 flight in April 2018.
High Throughput Analysis of Streptomycin Resistance in Soils

Background: Every year since 2016, 173,000 hectares of Florida citrus groves (1.2% of the land area of the state) are being treated with oxytetracycline and/or streptomycin for the control of citrus greening disease. The effect of these antimicrobials on antimicrobial resistance in non-target soil bacteria organisms is unknown. To rapidly assess changes in streptomycin resistance in soil microbial communities of citrus groves, a high throughput, barcoded sequencing approach was developed for the highly conserved rpsL gene, which codes for an essential protein in the small subunit of the ribosome. Single base mutations in this gene are known to confer streptomycin resistance in many bacteria.

Methods: Universal primers were designed to amplify that portion of the rpsL gene containing the alleles known to confer streptomycin resistance. Given that rpsL is not as highly conserved as the 16S rRNA gene, a set of primers is required for each bacterial phylum or sub-phylum. A set of primers were designed to amplify rpsL from the alphaproteobacteria. DNA samples from 15 soil samples was isolated and amplified using this primer set with a unique barcode for each soil sample. A database containing all sequenced rpsL genes in NCBI was constructed to identify as many of these sequences as possible.

Results: Over 13.6 million reads were obtained from the soils. Of those, 86.4% of reads showed no changes in the two codons typically responsible for streptomycin resistance. Single base mutations in codons 88 and 43 were identified in 13.04% and 0.55% of reads, respectively. These mutations are known to confer streptomycin resistance. These samples were taken from groves that had not yet been sprayed with streptomycin, giving us a baseline level of rpsL mutation prior to selection pressure being applied. More than 98% of reads were classified to alphaproteobacterial rpsL and were dominated by Bradyrhizobium, Sinorhizobium, Ensifer, Rhizobium, and Devosia. Conclusions: These results show that streptomycin resistance conferred by rpsL can be rapidly quantified using making it easier to assess the effects of streptomycin applications on non-target bacteria.
**Session Number:** 426  
**Session Type:** Late-Breaker Poster Presentations

Abstract Title:  
In-Gel Loop Mediated Isothermal Amplification (gLAMP) Enables Fast Coliphage Quantification in Environmental Waters  

Primary Author Block:  
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Abstract Body:  
Background: Pathogenic enteric viruses transmitted through environmental waters are the major cause of waterborne diseases. Direct monitoring of specific viral pathogens is impractical due to methodological limitations. In 2015, the US EPA initiated a criteria development process considering the use of coliphages as viral indicators of fecal contamination in ambient water. Traditional culture-based plaque assay requires 1 to 3 days to obtain the results. RT-qPCR provides a much shorter sample-to-result time (3 to 5 hours), but it is prone to inhibitions in environmental samples and can only be performed by trained personnel in well-equipped laboratory environment.  

Methods: We developed an in-gel loop mediated Isothermal amplification (gLAMP) assay. Viral particles (coliphage MS2) were immobilized with LAMP reagents within polyethylene glycol hydrogels, and then the viral RNAs were amplified through an in-situ LAMP reaction. Due to the restriction effect of hydrogels, one viral particle only produces one amplicon dot. Therefore, the sample virus concentration can be determined based on the number of fluorescent dots after the reaction using a smartphone.  

Results: gLAMP enables coliphage quantification within 30 min using simple lab devices (see figure). The gLAMP results correlated well with traditional plaque assay counts ($R^2=0.984$, $p<0.05$) and achieved similar sensitivity to RT-qPCR (1 PFU/reaction). Moreover, gLAMP demonstrated a high tolerance against inhibitors present in toilet wastewater, in which RT-qPCR was completely inhibited.  

Conclusions: Considering its simplicity, sensitivity, and rapidity, gLAMP holds great potential for microbial water quality analysis, especially in resource-limited settings.
Session Number: 426
Session Type: Late-Breaker Poster Presentations
Session Number: 426
Session Type: Late-Breaker Poster Presentations
Session Title: SUNDAY - AES Late-Breakers
Session Start Date Time: 6/10/2018 12:45:00 PM
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Session Primary Track: Applied and Environmental Science
Abstract Control Number: 7486
Poster Board Number: SUNDAY - AES LB11

Abstract Title:
Applied Biosystems 7500 Fast qPCR for simultaneous detection of Campylobacter jejuni, C. coli and C. lari

Primary Author Block:
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Abstract Body:
Background: Campylobacter jejuni, C. coli, and C. lari account for 95% of total Campylobacter infection cases. A SmartCycler-based multiplex qPCR was previously developed to simultaneously identify three target species. However, the SmartCycler instrument will be discontinued by its manufacturer, Cepheid, after December 2018. To address this issue, we have recently transitioned the qPCR platform to the Applied Biosystems 7500 Fast (AB7500F) system. Methods: Primer and probe sequences were evaluated for AB7500F, then qPCR conditions were optimized. Amplification efficiency and detection limit were determined using genomic DNA (gDNA) of Campylobacter ATCC strains as standards. Exclusivity and inclusivity were assessed using randomized and blinded gDNA from various sources including ATCC strains, published strains, as well as fully characterized isolates from archived collection. Results: A multiplex qPCR has been optimized for AB7500F. Primers and probes for C. jejuni and C. lari are the same as those used in the SmartCycler method. For C. coli identification, a new set of primers and probe was designed and it resulted in better qPCR efficiency than the set for SmartCycler. Under optimized conditions, the amplification efficiency of AB7500F qPCR is 90.9%, 86.5% and 94.6% for C. jejuni, C. coli and C. lari, respectively. This method is 100% species-specific when tested with gDNA from 86 target strains (50 C. jejuni, 27 C. coli, and 9 C. lari strains). There is no false positive signal on gDNA from 38 non-target ATCC strains. The limit of detection is four, seven, and two genome copies for C. jejuni, C. coli, and C. lari, respectively. Conclusion: There is no significant difference between the SmartCycler-based and AB7500F-based qPCR regarding efficiency, specificity and sensitivity. AB7500F-based qPCR will efficiently facilitate simultaneous detection for C. jejuni, C. coli, and C. lari after the projected discontinuation of SmartCycler.
Abstract Title:
Gallium Maltolate Reduces the Burden of Macrolide-Resistant Rhodococcus equi in Soil and Feces

Primary Author Block:
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Abstract Body:
The soil saprophyte Rhodococcus equi (R. equi) causes severe pneumonia in foals. Widespread use of macrolides to treat this pneumonia has been associated with increased prevalence of macrolide-resistant R. equi. To combat this problem there is a need for alternative antimicrobial therapies. Our laboratory has demonstrated efficacy of the gallium maltolate (GaM) against R. equi in vitro and in vivo. Both macrolides and GaM administered orally are shed in feces into the soil where R. equi persists, but their impact on macrolide-resistant isolates in soil is unknown. Thus, the objective of this study was to compare the proportion and concentration of macrolide-resistant R. equi (MRRE) in feces and soil exposed to macrolides with those exposed to GaM. To answer this question, 12 plots each with approximately 500 grams (g) of soil each inoculated with an isogenic MRRE strain and isogenic macrolide-susceptible R. equi (MSRE) strain at equal concentrations. Untreated soil (n = 4) was compared to soil treated with either 1 g of GaM (n = 4) or 1 g of erythromycin (n = 4) weekly; all soil had 5 g of equine feces added weekly. Quantitative culture performed initially, biweekly for 12 weeks and then at an interval of 4 weeks until 24 weeks, selecting for MRRE and MSRE strains using NANAT media. Groups were compared using linear mixed-effects modeling. Results indicated that GaM-treated soil had a significantly (P < 0.05) reduced number and proportion of MRRE following 4 weeks of treatment, although MSRE grew in both GaM-treated and untreated soil. Erythromycin-treated soil resulted in a decrease in total R. equi but a significantly (P < 0.05) increased proportion of MRRE than GaM-treated or untreated plots. We conclude that unabsorbed macrolide in fecal shedding would favor persistence of MRRE in the environment, whereas GaM would not. Suggesting that using GaM as an alternative to macrolides could decrease selection for MRRE in the environment at horse farms where foals suffer from R. equi pneumonia.
Abstract Title:
A path toward genetic manipulation of Megasphaera elsdenii
Primary Author Block:
E. Hatmaker, L. A. Riley, A. Guss; Oak Ridge Natl. Lab., Oak Ridge, TN
Abstract Body:
Background: As the energy sector shifts toward affordable and sustainable fuels, bacteria able to ferment sugars into longer carbon chain acids are of increasing interest to biofuel production models. Megasphaera elsdenii is a bacterium found in the rumen of cattle which produces hexanoic acid as well as butanoic acid. Due to the lack of genetic engineering tools for M. elsdenii, the underlying mechanism of production is currently poorly characterized. Engineering this strain for further use in biofuel production would be advantageous in the production of hexanoate and related compounds.

Methods:
We used PacBio sequencing generated by the Joint Genome Institute (JGI) to assemble complete genomes for two strains of M. elsdenii, NCIMB702410 and ATCC25940. ATCC25940 is synonymous to DSM20460, which has a draft genome available through NCBI. JGI also produced Illumina RNA-seq reads which we used to check functionality of restriction-modification (R-M) systems in both strains. Results: NCIMB702410 encoded one functional Type I R-M system whereas ATCC25940 encoded two Type I systems and one Type II. We circumvented the R-M systems of both strains to enable genetic transformation. We also compared gene content and sequence similarity, generated metabolic reconstructions, and compared the resulting metabolic models of the two strains to gain deeper understanding of the production of butanoic and hexanoic acids. Conclusions: Understanding the similarities between the two M. elsdenii strains, as well as having a solid genomic basis for each enables the development of genetic tools, as well as elucidating targets for modification.
Mechanistic Patterns of Soil Fungi control their Polyurethane Biodegradation Efficiency

Primary Author Block:
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Abstract Body:
Polyurethane (PU) is widely used in many aspects of modern life like medical, automotive and many other industrial fields because of its versatility and resistance. However, the slowly degraded PU waste generates major pollution problems for plants, wildlife and even human populations. Bioremediation of plastic waste is considered as an economic and environment friendly strategy for plastic waste management that could totally reduce the severe pollution produced from the conventional plastic remediation methods. Thus in the present study, we shed the light on the potential mechanisms that the soil fungi could employ to degrade the Impranil DLN as a liquid form of PU. Fungal strains were isolated from plastic contaminated soils and were characterized by various abilities in enzymes and acid production. Biodegradation experiments were performed using selected fungal isolates (Aspergillus sp., Aspergillus sydowii, Monascus sp., Aspergillus flavus, Lichtheimia ramosa, and Gymnoascus dankaliensis) and an interval monitoring was done for; changes in PU functional groups by Fourier transform infrared (FTIR) spectroscopy, estimating the complexation between fungal cells and PU by zeta potential, investigating the changes in surface and internal fungal cell structure by Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM), and measuring the hydrophobicity of the fungal cells. Our findings revealed that a wide range of enzymes (protease, esterase, lipase, laccase and polyurethanase) were produced by the isolated fungi during the biodegradation experiments and these results may demonstrate the important contributions of these isolates in plastic degradation. Moreover, we observed pronounced variation in the mechanistic patterns of the different fungal isolates affected their plastic biodegradation efficiency. Our results provide evidence that the fungal isolates (even for those of the same genus) were not limited solely to any single mechanism in the biodegradation process of PU, but were dependent on a number of interacting mechanisms such as absorption, enzyme/acid-based degradation and accumulation. However, most of the fungal isolates employed actively both the enzyme/acid-based degradation and absorption mechanisms in PU degradation. Our findings succeeded in describing how soil fungi interact effectively with PU and that will have great implications on developing novel approach of plastic bioremediation technologies to minimize the environmental pollution.
Assessing the Antimicrobial Activity of Copper-Coordinating Coatings

Primary Author Block:
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Abstract Body:
The use of antiseptics and disinfectants and their relationship to the rise of antimicrobial resistance are critically important topics in human and animal health. Due to its broad microbiological toxicity, copper has been used for thousands of years in antimicrobial applications, yet without the widespread generation of resistance. Copper is used to maintain sterile surfaces, sees extensive use as an agrochemical fungicide, and is incorporated into coatings for the minimization of marine biofouling. We have found, unexpectedly, that a series of new polymer components, originally intended as renewable film-forming additives, have demonstrated the ability to coordinate copper (II) and, therefore, may have applicability in antimicrobial coatings. Polyhydrazones derived from levulinic acid and adipic acid diacylhydrazide were successfully incorporated at 50% w/w into a commercial acrylic polymer (LR200).1 When exposed to an aqueous copper (II) nitrate solution, the modified coating, in turn, coordinated the copper, as evidenced by the rapid and retained surface color change. In order to assess the antimicrobial efficacy of such coatings, we employed industry standard assessment methods2, but found them to be erratic (no reproducibility between replicate assessments of a surface). This method has a binary output (antimicrobial versus not-antimicrobial) and relies on a 2-log variance of proliferation for significance.

We required a more robust method with multiple replicates readily achievable and with less of a digital output, providing information in a time series (e.g. at 4, 8 or 24 h). Consequently, we developed a new method for the assessment of microbial survival on surface coatings (Escherichia coli at 20-200 cell counts/cm², typical error ± 10% cell counts/cm² S.D n=4). The key modifications to the assay are the use of a 24-well plate to permit replicate data, the collection of data at multiple time-points, and the introduction of controls. The new method for testing the antimicrobial activity of a surface is an improvement upon the industry standard, and, therefore, the methodology is in place to quantitate, for the first time, the surface concentration of copper (II) that is required to confer antimicrobial activity. By quantifying the copper (II) coordinated at the surface of our coatings and employing the new methodology to determine antimicrobial activity (cells killed/cm² after time exposure), rechargeable antimicrobial coatings may be developed.
Antimicrobial Susceptibility of Lactic Bacteria Isolated from Amazonian Peruvian Fruits

Primary Author Block:
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Abstract Body:
Background: Lactic acid bacteria (LAB) are widely known for their probiotic properties however there has been growing concern about the possibility that LAB may be a source of antimicrobial resistance genes which could be horizontally spread to intestinal pathogens or native gut microbiota. Bacterial resistance to antimicrobials is increased by the misuse or prolonged use of these compounds, searching for LAB from natural sources and environments with unique characteristics such as the Peruvian Amazon could result in the isolation of safety strains. The objective of this study was to determine the antimicrobial susceptibility of LAB isolated from Amazonian Peruvian fruits as a first step to determine its probiotic potential. Methods: LAB strains were isolated from 14 Amazonian Peruvian fruits and species identification was based on sequence analysis of 16S rRNA genes. Antimicrobial susceptibility was evaluated by the disc diffusion method using amoxicillin (10 ug), ampicillin (10 ug), chloramphenicol (30 ug), vancomycin (30 ug), kanamycin (30 ug), sulfamethoxazole-trimethoprim (25 ug), streptomycin (10 ug), gentamicin (10 ug), tetracycline (30 ug), clindamycin (2 U), rifampicin (5 ug) and erythromycin (15 ug). Results: Based on 16S rRNA gene sequences the isolates were identified as Lactobacillus plantarum (26), Lactobacillus brevis (3), Weissella cibaria (5) and Weissella confusa (1). LAB strains showed high susceptibility (95% - 100%) to clindamycin, tetracycline, chloramphenicol, erythromycin, ampicillin, amoxicillin and rifampicin. While 98% to 100% of the strains were resistant to kanamycin, gentamicin, vancomycin, sulfamethoxazole - trimethoprim and streptomycin. It has been reported that resistance to these antimicrobials is intrinsic or natural in LAB and it has a minimal potential for horizontal spread. Conclusions: The results indicate that it is necessary to carry out further research to confirm the genetic nature of the observed resistance and to continue with the evaluation of the probiotic capacity of the isolated strains.
Session Title: SUNDAY - AES Late-breakers
Session Start Date Time: 6/10/2018 12:45:00 PM
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Session Primary Track: Applied and Environmental Science
Abstract Control Number: 7957
Poster Board Number: SUNDAY - AES LB9

Abstract Title:
Evaluation of Antimicrobial, Anti-biofilm and Disruption Efficacy of European Cranberrybush (Viburnum opulus L.) Fruit Juice against Pseudomonas aeruginosa Biofilm

Primary Author Block:
G. Ozan, F. Y. Ekinci; Yeditepe Univ., Istanbul, Turkey

Abstract Body:
Biofilm formation is one of the most crucial issue in food industry that causes several problems related with food safety, food spoilage, loss of production efficiency and consequential economic loss. Although many cleaning and disinfection strategies have been developed to combat biofilm problems in food industry, a more economical and environmental friendly control strategy is crucial to fulfill the need of industrial food safety. Pseudomonas aeruginosa is one of the most pathogenic strong biofilm producer bacteria causing spoilage in food products. European cranberrybush (ECB) (Viburnum opulus L.) fruits are rich source of phenolic compounds, and have shown to possess biological activities. In this study, the antimicrobial, anti-biofilm, and the disruption efficacy of ECB fruit juice on P. aeruginosa biofilm was assessed by using crystal violet staining method. Overnight culture of P. aeruginosa ATCC 15442 (107 cfu/ml) grown in TSB was incubated with varying concentrations of lyophilized ECB juice (200, 100, 50, 25, 10, 5, and 1 mg/ml) in a tissue cultured 96-well plate for 24 to 48 h at 37 oC. Samples without ECB juice served as control. P. aeruginosa was susceptible to ECB juice at high concentrations and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were found as 50 mg/ml and 100 mg/ml, respectively. Crystal violet staining results showed that sub-MIC and MIC concentrations (200-100-50-25 mg/ml) of ECB juice could significantly (90 %) reduce the biofilm formation by P. aeruginosa after 24 h of incubation, however anti-biofilm activity declined to 11 % with the decrease in concentration of juice. In contrast, disruption of preformed biofilm was more difficult to achieve and only 200 and 100 mg/ml ECB juice was partly effective on 24 h preformed biofilms. The present findings showed that ECB fruit juice might have a potential to reduce P. aeruginosa growth and biofilm formation in a concentration dependent manner.
Abstract Title:
Airborne Fungi in A Hosp. Drug Dilution Ctr. Before and after the Cleaning Process
Primary Author Block:
G. Link, A. Dias, L. B. Matter; Univ.e Regional Integrada do Alto Uruguai e das Missões, Santo Angelo, RS, Brazil
Abstract Body:
Advances in surgical and drug therapy have prolonged patients’ lives but a population more vulnerable to opportunistic infections has emerged. In the hospital environment, where there are highly susceptible people, fungi represent a problem because their resistance and easy dissemination. Periodic cleaning process and monitoring of the air quality comprises an important strategy to preventing infections. In this context, the objective of this work was to verify the quality of the air in a hospital drug dilution center in Brazil, before and after the cleaning weekly procedure with 2% hypochlorite. The evaluation of the fungi in the air was achieved by exposing Petri dishes with Sabouraud agar according to the 1/1/1 scheme (for 1 hour, 1 m above the floor and 1 m away from walls or another dish). After incubation for 25 °C for 5 days, CFU were quantified, isolated and incubated again for macroscopic and microscopic analysis. Sampling showed a contamination of 57 CFU (average of 14 CFU/plate) and 50 CFU (average of 12 CFU/plate) before and after cleaning process, respectively. The main fungi found before cleaning were: Aspergillus sp., Penicillium sp., Curvularia sp., Trichophyton sp. and Fusarium sp. whereas after cleaning were found Trichophyton sp., Fusarium sp. and yeasts. This work revealed that the air quality of the drug dilution center was compromised even after the cleaning process and the efficiency of the cleaning process as well as the products used should be constantly evaluated to ensure the effectiveness of the procedure.
Covert Rift Valley Fever in the Domestic Ruminant Populations in Uganda

Abstract Body:
Prior to the first recorded outbreak of Rift Valley Fever (RVF) in Uganda of March 2016, studies indicate presence of the RVF virus in Uganda without any overt outbreaks in either man or animals. Additionally, a number of isolates were also obtained, including the primordial isolate, from Semuliki Forest the mosquitoes of the Eretmapodite spp, for the Smithburn Modified Live Virus Vaccine (SMLVV) for animals. The first 2 datasets in this paper provide recent evidence, before March 2016, of the RVFV in Uganda, in select areas in the domestic ruminant populations. In 2013, sero-survey specimens from the districts of Hoima, Kibaale and Masindi were analyzed using a RVF inhibition ELISA and the results show an overall prevalence of 18.6% (12.5-26.7%) in the cattle population and 2.3% (0.4-12.1%) in the shoats population. In cattle, the prevalence was 12.1% (4.8-27.3%), 10.0% (2.8-30.1%), and 25.0% (15.8-37.2%) in the districts of Hoima, Kibaale and Masindi respectively while in shoats the prevalence in Masindi was 3.1% (0.5-15.7%), no antibodies could be detected, in shoats, in Hoima district. During an investigation in Agago and Kitgum districts of an undiagnosed illness in humans in 2011, a RVF sero-prevalence of 4.7% (1.3-15.5%) was detected in cattle while in shoats, it was 9.4% (3.2-24.2%). Both IgG and IgM antibodies to the RVF virus were detected at that time. These findings are synonymous to occurrence of disease during the ‘inter-epizootic periods’ in countries experiencing cyclic outbreaks. After the March 2016 RVF outbreak in Kabale, a planned multi-sectoral bio-surveillance pilot study was conducted in the outbreak district of Kabale and the surrounding ones. The study districts included; Kabale, Kanungu, Kasese, Kisoro and Rubirizi with the respective percentage (Positives / Animal numbers) data of 16.0% (83/520), 2.1% (4/193), 0.8% (1/130), 15.1% (21/139) and 2.7% (4/148). Of the 3 species investigated, the bovines exhibited the highest sero-prevalence of 15.2% followed by ovines with 5.3% and caprines with 4.0%. The study results are consistent with the confirmed outbreak in Kabale district and suspect cases in Kisoro district, in the domestic ruminant populations, around the same time period. The latter study is the most recent attempt to determine RVF sero-prevalence in Uganda, in many years. A more detailed study has been designed aimed at mitigating the risk of RVF in human and animal populations.
Abstract Title:
Animal Breed Composition Shapes Gut Microbiota, and its Effects on the Host Metabolic and Immunological Status

Primary Author Block:
P. Fan, L. Teng, C. Nelson, D. Driver, M. Elzo, K. C. Jeong;  Univ. of Florida, Gainesville, FL

Abstract Body:
Background: Great efforts have been made in breeding and genetic selection in the agricultural industry to generate animals with desirable traits. Host genetics can also influence the gut microbiota, which is considered a "second genome" that mediates host physiology. However, it is unclear how the gut microbiota is altered by the gradual changes of genetic composition, and its relationship with animal growth and health. Methods: To evaluate effects of genetic variation on gut microbiota, growth rate, and physiological status, we collected fecal and blood samples from 240 Angus-Brahman multi-breed cattle (n=240), with breed composition ranged from 100% Angus to 100% Brahman. Gut microbiota was detected by 16S rRNA gene sequencing, and analyzed by QIIME and PICRUSt. Metabolic and immunological status was evaluated by measurement of plasma glucose, triglyceride, and IgG1 levels. Associations among breed composition, gut microbial community and host phenotypes were assessed by Pearson’s correlation coefficient. Results: The gut microbial community structure linearly changed with a shift in genetic composition in the multibreed herd of Angus and Brahman cattle. Groups of bacterial families and genera with their presence and proportion linearly correlated with breed composition were identified. Growth rate was positively correlated with Angus proportion, and obese-associated Rikenecellaceae was enriched in fast-growing Angus cattle. Plasma IgG1 level decreased with Brahman proportion. The relative abundances of butyrate-producing bacteria Faecalibacterium, Blautia and Coprococcus, which are biomarkers of a healthy gut, increased in infection-tolerant Brahman cattle. Plasma glucose level increased with Brahman proportion. Bacteria in cattle with more Brahman proportion were predicted to contain more genes involved in carbohydrate metabolism but less related to bacterial infectious, while those with more Angus proportion were better involved in lipid biosynthesis. Associations between host phenotypes and microbial communities were also detected. Relative abundance of Faecalibacterium was positively correlated with growth rate. The plasma glucose level was positively correlated with relative abundances of Oscillospira, Bacteroides, [Prevotella], and Odoribacter. Conclusions: Our findings indicate that animal breeds modulate the structure and function of the gut microbiota, and thus the altered microbiota are closely associated with animal’s physiological and immunological status.
Abstract Title:
Alternatives to Antibiotics in Animal Agriculture

Primary Author Block:
K. Hoelzer, N. Wong, J. Thomas, K. Talkington; The Pew Charitable Trusts, Washington, DC

Abstract Body:
Background: The use of antibiotics in any setting contributes to the growing global threat of antibiotic resistance, so it is important to minimize the use of these drugs by eliminating unnecessary uses and finding other ways to prevent infections. In animal agriculture, non-antibiotic alternative products such as vaccines or probiotics play a crucial role in allowing farmers and veterinarians to reduce the use of antibiotics. Methods: A comprehensive literature review was conducted to summarize the options available to reduce the need for antibiotics in animal agriculture through the use of non-antibiotic alternative products. Academic veterinarians and food-animal experts with species-specific experience in clinical and extension work were consulted to provide feedback on the use of alternative products in the commercial setting and to confirm the findings from the literature search. Results: There is a body of scientific studies available that found promising results on the efficacy of some alternatives as growth promoters and, to a more limited extent, for use in disease prevention. Vaccines are among the most promising and widely used of these alternatives, but pre- and probiotics and other innovative products are also in use or currently being investigated. Many of these have been shown to simultaneously prevent infection and improve animal performance. To date, there are fewer options available for treatment. The effectiveness of alternative products can vary considerably by species and purpose of use. More research is needed to understand exactly why efficacy is so variable and to ensure optimized use. Alternative products also differ in how their use has to be timed to ensure effectiveness. A variety of other alternatives for growth promotion and/or disease prevention show positive early results, but more data under realistic conditions are urgently needed, as are data on potential interactions among alternatives. Conclusion: Overall, alternatives have the potential to replace antibiotics in many situations. Focused research and development will help bring promising technologies to the veterinary market and guide their use. That, in turn, will help reduce antibiotic use in animal agriculture without endangering animal health, productivity, and welfare.
Session Title: AES02 - Microbiology of Agricultural Systems: Microbiology of Animals and Aquaculture
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/10/2018 2:45:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 4660
Poster Board Number: SUNDAY - 780

Abstract Title:
Outstanding Abstract Award: Aeromonas Veronii, But Not A. Hydrophila, is the Main Cause of Cyprinid Fish Septicemia in Southern China: from Epidemiology to Key Virulence Determinant

Primary Author Block:
M. Xie, C. Ran, C. Qin, Z. Zhou; Feed Res. Inst., Chinese Academy of Agricultural Sci., Beijing, China

Abstract Body:
Motile aeromonad septicemia (MAS) caused by Aeromonas spp. often lead to high mortality and huge economic losses in aquaculture. The etiological agent of MAS has been mainly identified as A. hydrophila, with the importance of other Aeromonas species in the pathogenesis of MAS rarely investigated, and the mechanism of Aeromonas spp. infection not well-understood. Here, we analyzed the epidemiological data of cyprinid fish MAS in southern China from 2009-2014 and found that A. veronii infection was dominant (50.0%), followed by A. hydrophila mono-infection (20.8%) and mixed infection of the two species (16.7%). A number of isolates of the two species were tested in germ-free (GF) zebrafish larvae by the oro-intestinal infection route, and A. veronii strains showed consistently higher virulence than A. hydrophila. By screening transposon library in zebrafish larvae, the toxin aerolysin was identified as the key virulence factor for A. veronii. Consistent with the results in GF larvae, A. veronii showed lethality in an aerolysin dependent manner in adult zebrafish, while A. hydrophila cannot kill adult fish by the nature route. Histology and confocal microscopy results showed that aerolysin produced by A. veronii can disrupt the intestinal barrier of zebrafish, which enables the systematic invasion of not only A. veronii in the mono-infection, but also non-invasive A. hydrophila in the mixed infection. We further observed that the low virulence of A. hydrophila by natural infection route may be attributable solely to its inefficient aerolysin and the resultant defect in invasion, and complement or bypassing of this defect can lead to high virulence. Together, our data suggest that A. veronii is the main cause of septicemia in cyprinid fish in southern China and its aerolysin is responsible for barrier crossing to establish infection, which highlights the importance of A. veronii-targeted treatments in the future efforts against MAS.
Effect of PAHs and Iron on the Fitness of Tetracycline Sensitive Shigella Flexneri

Background: Urban rivers may be sources of antibiotics contamination that could support the spread of antibiotic resistant bacteria (ARB) to the population in case of contamination with antibiotics due to anthropogenic activities. Methods and Results: In an exercise to estimate this contribution, Shigella flexneri 2a YSH6000 (tetR) and S. flexneri 2a 1363 (tetS) were used in microcosms generated from Thames river (London, UK) from upstream and downstream of the city center. Filtered microcosms for each sector were enriched with tetracycline at lethal (10 µg/mL) and sub-lethal (10 ng/mL) concentrations and the fitness of an isogenic pair of Shigella flexneri 2a YSH6000 (tetR) and S. flexneri 2a 1363 (tetS) was then measured. In the presence of selective pressure in upstream microcosms, the resistant strain outcompeted the sensitive one, as expected. In contrast, sensitive S. flexneri tetS was found to significantly compete with resistant S. flexneri tetR at lethal concentrations of tetracycline in downstream microcosms. The concentration of polycyclic aromatic hydrocarbons (PAHs) benzo(a)pyrene, pyrene and phenanthrene was found to be 128, 171 and 128 times higher in downstream sector when compared to upstream sector, respectively. Also, iron was found to be significantly higher in downstream sector (7 µg/L) when compared with upstream. Further experiments showed that PAHs rendered the resistant S. flexneri tetR ~20% more sensitive to tetracycline. Sensitive S. flexneri tetS strain was able to persist at lethal concentration of tetracycline in downstream microcosms, at higher concentrations of PAHs. In addition, microcosms enriched with iron up to 70 µg/L showed that increments in iron concentrations reduced the growth of S. flexneri tetR in presence of lethal concentration of tetracycline. Conclusions: Our findings suggest that in a polluted river (PAHs and iron) sensitive S. flexneri cells may still thrive in presence of selective pressure. Fitness tests provide an additional tool to measure bioavailability.
Session Number: 429
Session Type: Poster
Session Number: 429
Session Type: Poster
Session Title: AES03 - Antimicrobial Resistance in the Environment: Antibiotic Activities and Resistance
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/10/2018 2:45:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 5535
Poster Board Number: SUNDAY - 782

Abstract Title:
Widely Used Disinfectants Can Promote Antibiotic Resistance

Primary Author Block:
M. Kim1, M. R. Weigand2, S. Oh3, J. K. Hatt1, R. Krishnan1, U. Tezel4, S. G. Pavlostathis1, K. T. Konstantinidis1; 1Georgia Inst. of Technology, Atlanta, GA, 2CDC, Atlanta, GA, 3Nanyang Technological Univ., Singapore, Singapore, 4Bogazici Univ., Istanbul, Turkey

Abstract Body:
Background: While misuse of antibiotics has clearly contributed to the emergence and proliferation of resistant bacterial pathogens with major health consequences, it remains less clear if the widespread use of disinfectants such as quaternary ammonium compounds (QAC) has contributed to this problem. Here, we provide evidence that exposure to benzalkonium chlorides (BAC), a widely used member of QAC, co-selects for antibiotic-resistant bacteria, and describe the underlying genetic mechanisms.

Methods & Results: BAC-fed bioreactors inoculated with river sediment selected for several bacterial taxa, including the opportunistic pathogen Pseudomonas aeruginosa, that were more resistant to several antibiotics compared to their counterparts in a control (no BAC) bioreactor. Metagenomics analysis of the bioreactor microbial communities, confirmed by genetic manipulations of derived isolates, suggested that integrative and conjugative elements encoding a BAC efflux pump together with antibiotic resistance genes were mainly responsible for these results. Further, exposure of the P. aeruginosa isolates to increasing concentrations of BAC selected for mutations in pmrB (polymyxin resistance) and physiological adaptations, which contributed to higher tolerance to polymyxin B.

Conclusions: Collectively, our results demonstrate that disinfectants can promote antibiotic resistance via several mechanisms, and highlight the need to remediate (degrade) disinfectants in non-target environments to further restrain spread of antibiotic resistant bacteria.
Abstract Title:
Comparative Study of the Ecological Risks of TiO2 Nanoparticles and Biocides on Aquatic Bacteria

Primary Author Block:

Abstract Body:
Biocides and nanoparticles are known for their antimicrobial properties and controlling of microbial growth. While effective at killing microbes, biocide use has the potential to stimulate antibiotic resistance in bacteria. Nanoparticles show great promise in a number of industrial applications beyond microbial growth control. The environmental fate of nanoparticles and biocides is poorly understood. The current study investigated the ecological risks of TiO2 nanoparticles and biocides on aquatic bacteria. The increased use of these chemicals necessitates that the gap in the information of the impact of biocides and nanoparticles on the environment. For this purpose, we are investigating the antibacterial effects of long-term exposure to biocides and nanoparticles, their impact on the microbial community composition, and the mechanism of toxicity of both compounds. Two chemicals were compared in this study, commercial samples of 99.9% titanium dioxide nanoparticles and Glutaraldehyde, 25%. Samples from various freshwater bodies were treated with varying levels of both TiO2 Nanoparticles and Glutaraldehyde and incubated for two weeks. Samples were collected for microscopic cell counts, 16S rRNA gene sequencing, and cytotoxicity measurements. The microbial community was profiled with PCR amplification and 16S rRNA genes analysis to observe the effect on the bacterial cells. For both nanoparticles and Glutaraldehyde specimens we expect, significant results for antimicrobial activity for varying concentrations. We look to see dramatically decrease in bacterial communities with increased concentrations of nanoparticles (0.25%, 0.50%, and 1%). Additionally, the impact of biocides on freshwater streams demonstrated that different biocides select for different microbial community compositions. This suggests that resistance to different biocides may be catalyzed by a distinct mechanism. As Biocides are more likely to select for antimicrobial resistant bacteria than nanoparticles, we would like to investigate the relative efficacy and impact on microbial community composition in freshwater settings. This may provide more insights into the mechanism of action and the biological mechanisms of antimicrobial resistance.
Abstract Title: Ciliates Promote the Interactive Transfer of Plasmid Encoding BlaNDM-5 between Human Pathogenic Escherichia coli and Environmental Aeromonas Caviae

Abstract Body: Multidrug-resistant (MDR) human pathogenic bacteria is a major concern to hospital or community-acquired infections. However, “hot spot” supporting effective gene transfer among bacteria remains undetermined. Meanwhile, ciliates (Tetrahymena) can facilitate the transfer of plasmid encoding NDM-β-lactamase between Escherichia coli strains by conjugation via vesicle accumulation (Okubo et al, IJAA 2017). We therefore speculate that ciliates can be considered as a hot spot for fostering the interactive plasmid transfer between human pathogenic bacteria and environmental bacteria. Against the background, we assessed if ciliates could promote the interactive transfer of plasmid encoding blaNDM-5 between human pathogenic E. coli and environmental Aeromonas caviae. Conjugation experiments were performed according to the following method. In brief, equal numbers (10^8 CFU/mL) of donor (D) and recipient (R) bacteria were mixed in PAS with or without ciliates (10^4 cells/mL) and were incubated overnight at 30 °C. Transconjugants (Tc) were detected on agar plate containing CAZ (10 mg/L), and the presence of carbapenem resistance determinants was also confirmed by PCR. The gene transfer frequency was expressed as the number (CFU) of Tc per R. As a result, in contrast to the absence of ciliates, ciliates can transfer the blaNDM-5-plasmid from E. coli TC170328 (CAZ >128mg/L) to Aeromonas no.86 (CAZ 1mg/L) with a frequency of 10^-5. The Aeromonas no.86 Tc showed MIC of CAZ with >128mg/L. Also, in contrast to the absence of ciliates, ciliates can take back the plasmid from the Aeromonas (CAZ >128mg/L) to other E. coli J53 (CAZ 0.5mg/L) with a frequency of 10^-4. The plasmid transfer was also confirmed by PCR amplifying blaNDM-5 gene. Sequestering D from R bacteria by transwell caused a significant decrease of the plasmid transfer, and distinct colored D and R bacteria were well accumulated in a vesicle of ciliate. Thus, ciliates can promote the interactive transfer of plasmid encoding blaNDM-5 between human pathogenic E. coli and environmental Aeromonas caviae, providing us valuable findings to control MDR human pathogenic bacteria from the viewpoint of “One health approach”.

Session Number: 429
Session Type: Poster
Session Title: AES03 - Antimicrobial Resistance in the Environment: Antibiotic Activities and Resistance
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Session Primary Track: Applied and Environmental Science
Abstract Control Number: 5205
Poster Board Number: SUNDAY - 786

Primary Author Block:
M. Matsushita1, T. Okubo1, J. Matsuo1, S. Nakamura2, H. Yamaguchi1; 1Hokkaido Univ., Sapporo, Hokkaido, Japan, 2Juntendo Univ., Tokyo, Japan
Abstract Title:
Recovering Whole Sequence of Class 1 Integrons in Environments by Constructing A Comprehensible Class 1 Integrase Database and Designing New Pcr Primers

Primary Author Block:
A. N. Zhang, Y. Yang, T. Zhang; The Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract Body:
Class 1 integrons are the major contributors to the acquisition and dissemination of antibiotic resistance genes (ARGs). However, comprehensive knowledge of their distribution and diversity in different habitats is lacking to evaluate their significance. A new integrase database was constructed integrating bacterial genome database (55,000 complete and draft genomes) and NCBI nr database (Figure 1). Integrases were firstly identified by keyword search and curated by sequence-based alignment plus phylogenetic topology. To amplify the whole sequence of class 1 integrons by PCR, the intI1 and sull were selected as the targets for primer design. The newly-designed primer pair was evaluated against the previous primers in terms of both the coverage and specificity by the results of Illumina Hiseq sequencing and Pacbio sequencing. Finally, the class 1 integrons in different human-related habitats were studied using the new primer pair. The intI1 database constructed here provided good references for primer design since it expanded the previous databases by 40%. It was evaluated that the previous PCR primers may only detect 25% of this new intI1 database. Compared to previous primers, the new primers could cover the whole sequence of class 1 integrons to comprehensively study their diversity, structure and gene cassettes. By exploring the class 1 integrons in different habitats, it was intriguing that the class 1 integrons in all habitats only carried ARGs of 55 genotype, with resistance to 7 types of antibiotics, i.e. aminoglycosides, sulfonamide, trimethoprim, beta-lactam, chloramphenicol, quinolone and macrolide-lincosamide-streptogramin. These observations implied that the high co-occurrence of intI1 and ARGs of other antibiotics could be caused by their co-enrichment and co-localization on the same transposons or plasmids. This may suggest that the significance of class 1 integrons might be limited on the acquisition and dissemination of ARGs.
Abstract Title:
Effect of Mutations in Penicillin-Binding Proteins of Clostridium Perfringens on their Affinity to β-Lactams

Primary Author Block:
M. Park, F. Rafii; Natl. Ctr. for Toxicological Res., Jefferson, AR

Abstract Body:
Background: In Clostridium perfringens, seven genes have been designated as putative penicillin-binding proteins (PBPs). We previously showed that β-lactam resistance development in C. perfringens 13124 resulted in the induction of mutations in one or more annotated genes for PBPs in strains resistant to penicillin, cephalothin and ceftriaxone. In this study, the effect of mutations on the affinity of the altered PBPs to different β-lactams was investigated. Methods: The relative binding of PBPs in the wild type 13124 and the penicillin-resistant mutants 13124PGR, cephalothin-resistant 13124CFR and ceftriaxone-resistant 13124TXR to the fluorescent penicillin derivative, Bocillin FL in the wild type and the mutants before and after incubation with different β-lactams was measured. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the membrane proteins, the PBPs were visualized by scanning fluorescent images of the gels. Results: Mutations in the gene for PBP2 (CPF_2395), a high molecular weight transpeptidase, resulted in decreased affinity of the altered CPF_2395 by 81% in 13124PGR, 65% in 13124CFR and 63% in 13124TXR. Treatment of the wild type and β-lactam-resistant strains with different β-lactams (cephalothin, penicillin, ceftriaxone, cefoxitin, and ampicillin) reduced the binding of CPF_2395 and various other PBPs to Bocillin-FL in the wild type and resistant strains. The affinity of PBPs CPF_2061, which is a D-alanyl-D-alanine carboxypeptidase for Bocillin-FL, was substantially intensified in all three mutant strains. Conclusions: It appears that various mutations observed in the gene for CPF_2395 in resistant mutants had decreased the affinity of the altered PBP2, CPF_2395, for β-lactams, and that the Gly871Cys substitution had the highest effect. The higher affinity of CPF_2061 for β-lactams in the resistant mutants may indicate changes in the resistant strains, including upregulation of CPF_2061, to compensate for the effect of decreased transpeptidase activity of CPF_2395 during peptidoglycan synthesis.
Abstract Title:
Whole-Genome Analysis of Extended-Spectrum β-Lactamase Escherichia coli from Different Animal Sources

Primary Author Block:
A. Ibekwe; US Salinity Lab., Riverside, CA

Abstract Body:
Background: Antimicrobial resistance associated with the spread of plasmid-encoded extended-spectrum beta-lactamase (ESBL) genes, conferring resistance to third-generation cephalosporins is a major concern in global public health. The purpose of this study was to use whole-genome sequencing to compare antimicrobial-resistant (AMR) of ESBL-encoding genes identified in Escherichia coli isolated from swine, beef, dairy, and poultry collected from different regions of the United States. Methods: Three hundred samples were typed into different phylogroups, characterized by REP-PCR and PCR was performed to identify the corresponding ESBL genes followed by susceptibility testing by the disk diffusion method. A total of 20 E. coli isolates were confirmed as ESBL producers by double-disk synergy testing and multidrug-resistant (MDR) to at least three antibiotics. Whole-genome sequencing was performed to identify the corresponding ESBL genes on the 20 isolates. Data were compared using WG-MLST and WG-SNP-based phylogenetic approaches. Results: Of the 300 isolates, 59.7% were resistant to sulfosoxazole, 49.3% to tetracycline, 32.3% to cephalothin, 22.3% to ampicillin, 20% to streptomycin, 16% to ticarcillin, and the remaining 12 antimicrobials carried less than 10% resistance. E. coli were identified into different sequence types (ST) including ST131 (n=6), ST405 (n=4), and other STs (ST10, ST58, ST393, ST 617, and ST2450). About 50 different resistance determinants, including acquired resistance genes and chromosomal resistance mutations were detected. Other ESBL genes identified included blaCTX-M, blaTEM, blaOXA, and blaSHV in 25.3%, 22.3%, 0%, and 13% of these isolates, respectively. Conclusions: Significantly, higher numbers of ESBL-E. coli were detected in swine and dairy manure than from any other animal sources, suggesting that ESBL E. coli may be more abundant in swine and dairy than other animal sources.
Abstract Title:
Phenotypic and Genotypic Characterization of Three Antibiotic and Metal Resistant Aquatic Bacteria Isolated from the Gut of the Mummichog Fish (Fundulus heteroclitus)

Primary Author Block:
N. A. Lloyd1, T. Barkay1, S. Nazaret2; 1Rutgers Univ., New Brunswick, NJ, 2French Natl. Ctr. for Scientific Res., Villeurbanne, France

Abstract Body:
Exposure to metals may promote multi-drug resistance (MDR), a potent public health concern, through co-selection of antibiotic and metal resistances. Co-selection occurs through 1) co-resistance (resistance genes are co-located in the genome, or 2) cross-resistance (one process, such as toxicants removal by efflux pumps, mediates both resistances). Here, we investigated the mechanisms underlying the association between MDR and metal resistance in three bacterial strains isolated from the gut ingesta of the mummichog fish (Fundulus heteroclitus). We sequenced the genomes of the three strains to distinguish the roles of co-resistance from cross-resistance in observed phenotypes. The genome of Shewanella BC20, a strain resistant to carbapenems, penicillins, cephalosporins, and vancomycin, contained 51 resistance gene homologs including the intrinsic resistance genes blaOXA-48 and a class C β-lactamase, which are of public health concern. The genomes of two Vibrio strains, T9 and T21, contained 99 and 109 resistance gene homologs, respectively, and were resistant to penicillins and aminoglycosides. They harbored quinolone resistance genes (qnrS) and class A carbencillin-hydrolyzing β-lactamase (CARB) genes, which confer resistance to penicillins. Interestingly, the Vibrio spp. carried resistance genes on genomic islands. All three strains were resistant to arsenate, and harbored arsenate resistance genes (arsC, arsR, ACR3). The Vibrio spp. were resistant to cadmium, and all three strains contained various metal efflux specifying p-type ATPases (e.g., cadmium and copper ATPases). Vibrio T21 and Shewanella BC20 were resistant to mercury, but lacked resistance genes (mer). One 20kb region in T9 contained zntA, a P-type ATPase, multidrug transporter (acrB), and efflux transporter genes (e.g., mexE) but no genetic linkage between antibiotic and metal resistance genes was observed in strains T21 and BC20. Importantly, genes encoding efflux pumps were identified in all genomes, including MFS transporters (e.g. Bcr/CflA), and multidrug ABC transporters (e.g. emrD). Thus, genomic analysis points to cross-resistance, rather than co-resistance, as the main mechanism of co-selection of metal and antibiotic resistances in our strains suggesting that exposure to metals may affect a pool of resistance genes in aquatic environments. These results hold both environmental and clinical importance, as some of the antibiotic resistance genes identified in these organisms were previously shown to occur in human pathogens.
Abstract Title:
HARBORING A FUGITIVE: IDENTIFYING UNEXPLORED VECTORS AND CARRIERS OF ANTIBIOTIC RESISTANCE PLASMIDS

Primary Author Block:
L. E. Brooks, M. Kaze, M. Sistrom; Univ. of California, Merced, Merced, CA

Abstract Body:
Background: Antibiotic resistance in pathogens is a globally significant problem that is exacerbated by horizontal gene transfer. Despite intervention methods aimed at decreasing the prevalence of antibiotic resistance in pathogens, resistance remains on the rise leading to an increase in both hospital and community acquired infections. To better understand the full picture of this growing health crisis, the role of plasmid exchanges in the environment needs to be considered. Exploring the environmental resistome and looking for potential vectors or carriers of antibiotic resistance plasmids is a daunting task but can be started by identifying plasmids or bacterial groups of interest that may warrant further investigation. Methods: To accomplish this first step, we used publicly available bacterial plasmid sequences reported in complete genome assemblies to curate a comprehensive plasmid database. We then used this database to search for broad host range plasmids found in a number of taxa. Additionally, we identified environmental bacteria that may be acting as vectors or carriers capable of harboring clinically-relevant plasmids. Results: Our analyses revealed that nearly one-third of the plasmid sequences from the database were found in multiple isolates. While some plasmids were specific to certain taxonomic groupings, broad host range plasmids, found in different taxonomic groupings were also detected. Additionally, species capable of harboring a variety of different plasmids were identified, and their potential for exchanging plasmids with bacterial groups of interest assessed. This revealed a number of environmental bacteria that may be acting as vectors of clinically relevant plasmids. Conclusions: This work provides a starting point to enable future research into the role of environmental bacteria in genetic exchange with pathogens.
Abstract Title:
Isolation, Screening and Performance Studies of Mycelium Forming Actinomycetes from Fallow Nigerian Agricultural Soil with Bioactive Compounds against Typed Clin. Bacteria

Primary Author Block:
J. Olaitan, S. B. Akinde, F. Aseyemi, A. Yusuf, A. Bankole; Osun State Univ., PMB 4494, Osogbo, Nigeria, Osogbo, Nigeria

Abstract Body:
A total of 158 mycelium forming Actinomycetes strains were isolated from a fallow Nigerian agricultural soil over a period of one year. Preliminary screening of all the isolates was done using perpendicular streak method against five Gram negative and three Gram positive typed clinical bacterial isolates. All the 26 promising Actinomycetes strains were identified using their morphology, microscopy and biochemical characteristics. Bioactive compounds were extracted from the four most potent strains ACT 06, ACT 10, ACT 11 and ACT 19 using submerged state fermentation method and characterised using FTIR. Activity against the typed clinical isolates was by agar well diffusion method. Presumptive identity of the 26 promising Actinomycetes isolates revealed Actinomyces sp. (61%), Nocardia sp. (4%), Rhodococcus sp. (4%) and Streptomyces sp. (31%). All the four most potent strains were Streptomyces sp. and were active against at least one of the test organisms. The ACT 06, ACT 10, ACT 11 and ACT 19 strain showed activity against Pseudomonas aeruginosa ATCC 10145 (6 - 14 mm) and Corynebacterium diphtheriae ATCC 13812 (8 - 23 mm) whereas only ACT 06 showed activity against Bacillus subtilis NCTC 8263 (24 mm). None of the strains was active against Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 43816, Proteus mirabilis ATCC 7002, Salmonella typhimurium ATCC 14028 and Staphylococcus aureus NCTC 6571. FTIR analysis showed the presence of functional groups OH, COOH, C=C, C-H and NH2 in the compounds present in the extracts. All the compounds belong to non-heterocyclic group.
Abstract Title: Screening of Soil Microbial Isolates for Antibiotic Activity
Primary Author Block: H. Sahinoglu, P. Thomase, N. Daniels, C. Ingram, B. Okeke; Auburn Univ. at Montgomery, Montgomery, AL
Abstract Body: Antibiotic resistance by pathogenic microorganisms spurred increasing research on new antibiotic substances. Reasons that account for antibiotic resistance by microorganisms include genes for enzymes that inactivate antibiotics, ejection of the antibiotic by plasma membrane proteins, and mutations affecting mode of action of the antibiotic. This research attempts to isolate potent antibiotic producing bacteria from soil. The soil samples were collected from Prattville, AL, North-Montgomery, AL, and around the AUM campus in Montgomery, AL. Three random soil samples were collected from each area and pooled. Nineteen tentative antibiotic producing isolates were purified by repeated streaking on tryptic soy agar plates. After further screening by agar plate assay, four isolates, N-1, P-2, P-12, P-13, were selected as antibiotic producers. Isolate P13 strongly inhibited Staphylococcus aureus and S. epidermidis and slightly inhibited Citrobacter freundii and Alcaligenes faecalis on agar plates. DNA based techniques were employed to characterize the selected isolates. BLAST analysis of ribosomal RNA gene sequence of P-13 revealed similarity to Bacillus amyloliquefaciens, B. siamensis and B. methylotrophicus. Production of antibacterial substance by P-13 in liquid culture is being studied.
Abstract Title:
Effects of Glyphosate, A Common Herbicide, on Farm Animal-Associated Enterobacteriaceae

Primary Author Block:
O. Makarova, J. Poeppe, K. Bote, U. Roesler; Freie Univ.et Berlin, Berlin, Germany

Abstract Body:
Residues of glyphosate, the most used herbicide in the world, are commonly found in the environment and food supply chain. Recently, its effects on microorganisms and antibiotic resistance have been recognised, raising concerns about the effects of glyphosate in animal feed on microbiome. The objective of this study was to investigate the actual levels of resistance to glyphosate in diverse isolates of farm animal-associated Escherichia coli and Salmonella serovars, and its ability to induce resistance. Minimal inhibitory concentrations (MIC) of glyphosate and the glyphosate-containing herbicide product Roundup™ were determined using a broth microdilution method for 120 Salmonella and 238 E. coli isolates. An evolve-and-resequence experiment was performed on two isolates of E. coli (with and without an ESBL marker). Despite a relatively narrow overall distribution of MIC in both species (Salmonella: 40-80 mg/ml for glyphosate and Roundup™; E. coli: 5-10 mg/ml for glyphosate, 20-40 mg/ml for Roundup™), there were small but significant differences between certain groups of isolates depending on the pathogenicity profile, host species, ESBL status or time of isolation. Resistance induction response to Roundup™ was similar for ESBL and non-ESBL E. coli strains, with early extinctions of bacterial populations at 2x MIC. For glyphosate, the ESBL strain was also unable to grow at concentrations >MIC, whereas the non-ESBL strain readily adapted to growth at 2-4x MIC of glyphosate. Our results demonstrate that Salmonella are more resistant to glyphosate and Roundup™ than E. coli, and that despite an overall narrow distribution of MIC, certain characteristics (pathogenicity, host species, ESBL status or time of isolation) show a small but statistically significant association with an increased glyphosate resistance. Additionally, we found differences between glyphosate and Roundup™ and individual bacterial strains in the ability to induce resistance. Although glyphosate resistance does not occur easily, it is possible to select for increased tolerance. Further investigations are needed to determine the relevance of these findings to animal husbandry in one health context.
Abstract Title:
Polyhydroxyalkanoates Synthesis by Bacillus Aryabhattai C48 Isolated from Cassava Dumpsites in South-Western Nigeria
Primary Author Block:
T. O. Salaam1, N. Jamil2, A. K. Lawal1; 1Federal Inst. of Industrial Res., Oshodi, Lagos, Nigeria, 2Univ. of the Punjab, Lahore, Pakistan
Abstract Body:
Background: Environmental and health problems caused by the presence of persisting synthetic polymers of plastics necessitates the use of eco-friendly bio-polymers as alternatives in plastic production. Polyhydroxyalkanoates (PHAs) are bio-polymers made by a wide group of bacteria under carbon rich and nutrient limiting (such as nitrogen) growth conditions. Their physical and chemical properties are comparable with synthetic polymers such as polyethylene & polypropylene and are thus suitable alternatives in plastic production. PHAs also have vast biomedical (nerve engineering & bone scaffolding) and packaging applications. We present findings from the use of a newly isolated bacterium for sustainable PHAs production. Methods: The organism was screened for PHAs production in a carbon rich medium (2% - glucose; glycerol; starch; sugarcane molasses) using the viable colony (0.5µg/ml of Nile Red and Nile Blue A) and Sudan Black B (0.3 %) staining methods. The bacterium was identified by 16SrRNA sequencing. Production of PHAs was achieved using fresh cultures of bacterium in a nitrogen limiting medium supplemented with 2% glucose over 96 hours. The experiment was repeated using glycerol, starch and sugarcane molasses as carbon sources. PHAs were extracted from lyophilized bacterial biomass by sodium hypochlorite/chloroform method. Extracted PHAs were analyzed by FT-IR for the detection of functional groups. The organism’s PHA synthase genes, PhaC & PhaR were also partially amplified and sequenced. Results: The organism was identified as Bacillus aryabhattai C48 and produced orange and yellow fluorescence for all carbon sources used indicating the presence of PHAs. Blue black intracellular bodies of PHAs were also detected with Sudan Black B. Growth curves showed greatest biomass accumulation in 2% starch medium. B. aryabhattai C48 achieved PHAs production of 17 % in starch, 11 % in glucose, 13 % in glycerol and 10 % in sugarcane molasses at 24, 72, 96 and 48 hours respectively. FT-IR spectra showed peaks at 1276 cm⁻¹, 1229 cm⁻¹ & 1183 cm⁻¹ corresponding to P3HB & P3HB3HV. Detection of peaks at 1721 cm⁻¹ & ranging from 1500 - 800 cm⁻¹ reveal conformational changes of mcl-PHA & scl-mcl PHA in crystalline and amorphous phases. PhaC & PhaR sequences showed maximum homology with B. aryabhattai B8W22. Sequences of 16SrRNA, PhaC and PhaR have been deposited in the NCBI GenBank with accession numbers KY855373.1, KY855379.1, MF947450.1 respectively. Conclusions: The results show that B. aryabhattai C48 has great potential for sustainable PHAs production.
Abstract Title:
Simultaneous Hydrolysis and Fermentation Using Potato-Peel Waste to Bioethanol by Co-Cultures of Indigenous Strains of Aspergillus and Pichia

Primary Author Block:
S. Yahya; Univ. of Karachi, Karachi, Pakistan

Abstract Body:
Background: The abundance of starch on earth provides a rich source of energy for all life forms. Starch is edible, water soluble and easy to hydrolyze compared to cellulose especially for bioethanol production. In recent years, the paradigm of separate hydrolysis and fermentation for bioethanol production is shifted to simultaneous hydrolysis and fermentation (co-culture system). Co-culture of an amylolytic and an ethanol producing microorganism is used to convert starch into sugar followed by its conversion to ethanol in a single step. Methodology: Two indigenous fungal strains, A. niger MS 101 (KX243269) and A. tubingensis SY 1 (KX243270), with raw starch-digesting ability and a yeast strain Pichia kudriavzevii SY 11 (KX363848) with higher ethanol producing ability were identified by ITS sequencing and sequences submitted to NCBI gene bank. Starch hydrolysis and ethanol production was carried out by simultaneous hydrolysis and fermentation (SmHF) as well as separate hydrolysis followed by fermentation (SHF) at 30oC for 7 days under submerged fermentation (SmF) and solid-state fermentation (SSF) conditions using potato-peels waste (PPW) as a substrate. The co-culture combinations used were A. niger MS 101 + Pichia kudriavzevii SY 11 and A. tubingensis SY 1 + Pichia kudriavzevii SY 11. Ethanol was recovered by distillation and quantified by potassium dichromate method. Results: Bio-ethanol production by co-culture (i.e. simultaneous hydrolysis and fermentation of potato-peels) is more suitable compared to separate hydrolysis following fermentation. Under SSF, the combination of A. niger MS 101 and Pichia kudriavzevii SY 11 yielded 6 g/Kg ethanol in 120 h. On the other hand co-culture of A. tubingensis SY 1 and Pichia kudriavzevii SY 11 yielded ~4 g/Kg ethanol after 120 h under submerged conditions. Conclusions: Co-culture approach provides an alternate, time-saving and cost-effective method for the biodegradation of industrial waste along with appreciable yield of energy, as fuel.
Abstract Title:
Metatranscriptomic Analysis of A Medium Chain Fatty Acid Producing Microbiome

Primary Author Block:
M. J. Scarborough, C. E. Lawson, J. J. Hamilton, T. J. Donohue, D. R. Noguera; Univ. of Wisconsin - Madison, Madison, WI

Abstract Body:
Background: Medium-chain products have many industrial uses. Renewable bio-production of medium-chain fatty acids (MCFA) has been proposed to reduce petroleum demands. In this study, we utilized a microbiome to produce MCFA from a lignocellulosic conversion residue generated at the Great Lakes Bioenergy Research Center. We used metatranscriptomics to identify roles of community members and propose strategies to improve production of MCFA. Methods: A bioreactor fed switch grass ethanol refining residue was operated for 120 days. The reactor converted residual xylose and complex carbohydrates to acetate, butyrate, hexanoate and octanoate. Metagenomes derived from five reactor samples sequenced with the HiSeq (2x250bp) platform were assembled with Metaspades and binned with MaxBin. Draft genomes were annotated using Metapathways and multiple annotation databases. We mapped cDNA reads to the draft genomes to identify expressed genes.

Results: The recovered draft genomes were related to Lactobacillus (LAC), Pseudoramibacter (PSE), Lachnospiraceae (LCO), and Coriobacteriaceae (COR). Highly expressed genes indicate that multiple LAC organisms consume both hexose and pentose carbohydrates, producing lactate and acetate via the phosphoketolase pathway. Three COR organisms consume hexoses, producing lactate, acetate, and hydrogen gas. LCO and PSE express genes required for MCFA production via reverse-β oxidation. Based on thermodynamics, we hypothesize that LCO produces butyrate from xylose and PSE produces hexanoate and octanoate from endogenous lactate. Conclusions: In total, the community structure in Figure 1 is proposed. This work also identifies potential strategies to increase production of octanoate including (1) utilizing a synthetic community of lactate-producing Lactobacilli and a lactate consuming MCFA producer; and (2) intermittent control of the hydrogen partial pressure to drive elongation of acetate and butyrate to longer products.
Abstract Title:
Recruiting Energy-Conserving Sucrose Utilization Pathways for Enhanced Biochemical Production in Bacillus

Primary Author Block:
J. Feng1, Y. Gu1, C. Song2, Y. Wang1; 1Auburn Univ., Auburn, AL, 2Nankan Univ., Tianjin, China

Abstract Body:
Background: Sucrose is a naturally abundant and easily fermentable feedstock for various biochemical production processes. By now, several sucrose utilization pathways have been identified and characterized. Among them, the pathway consists of sucrose permease and sucrose phosphorylase is an energy-conserving sucrose utilization pathway because it consumes less ATP when comparing to other known pathways. Methods: Four combinations of the energy-conserving sucrose utilization pathways consisting of the sucrose permease gene (cscB from Escherichia coli or cscB from Bifidobacterium lactis) and the sucrose phosphorylase gene (sucP from Bifidobacterium adolescentis or gtfA from Streptococcus mutans) were introduced into Bacillus amyloliquefaciens 3Δ strain (a derivative of NK-1 strain with the deletion of its native sucrose utilization pathway) and Bacillus subtilis FJ-1 strain to improve the production of γ-PGA and 2,3-butanediol (2,3-BD), respectively. Results: Results demonstrated that the combination of cscB from E. coli and sucP from Bifidobacterium adolescentis showed the highest sucrose metabolic efficiency in B. amyloliquefaciens. The corresponding mutant 3Δ-CES consumed 49.4% more sucrose and produced 38.5% more γ-PGA than the control NK-1 strain under the same fermentation conditions. The combination of cscB from E. coli and gtfA (encoding sucrose phosphorylase) from Streptococcus mutans showed the most remarkable enhancement of 2,3-BD production in B. subtilis strains. With sucrose and sugar cane juice as substrate, respectively, the FJ-1-CEG strain produced 23.8% and 44.5% more 2,3-BD than the control FJ-1 strain. Conclusions: To our best knowledge, this is the first report concerning the enhancement of the target product production by introducing the heterologous energy-conserving sucrose utilization pathways in Bacillus strains. Such a strategy can be easily extended to other microorganisms for reinforced biochemical production using sucrose as substrate.
Abstract Title:
Auxins: A Potential Modulator for Cell Growth and Lipid Accumulation in Microalgae Chlorella Emersonii and Scenedesmus Opoliensis

Primary Author Block:
J. Singh, N. Chakravarty, P. Shukla, R. P. Singh; Indian Inst. of Technology Roorkee, Roorkee, India

Abstract Body:
The biodiesel production from microalgae is predominantly related to higher biomass yield and lipid content. Plant hormones are the chemicals synthesized by the plants, which play a crucial role in regulating the growth and development. The present work elucidate the impact of auxins mainly indole-3-acetic acid (IAA), indole-3-propionic acid (IPA) and indole-3-butyric acid (IBA) on the biomass, chlorophyll content, lipid content and lipid productivity of the microalgal strains i.e. Chlorella emersonii and Scenedesmus opoliensis. Amongst auxins, IBA and IAA had resulted into 39 % and 35 % increase in the lipid content respectively. In addition, both the auxins triggered increased biomass production and 1.5-2.0 fold increase in extractable chlorophyll content. Further, treatment of Chlorella emersonii with IBA (5.0 mg L-1) and IAA (20 mg L-1) had led into comparatively higher lipid productivity of 42 mg L-1 day-1 and 50 mg L-1 day-1 respectively, whereas the lipid productivity obtained with Scenedesmus opoliensis was 30 mg L-1 day-1 and 33 mg L-1 day-1 under similar conditions. Therefore, increased biomass and lipid productivity obtained using auxins denoted these as a potential regulator for improving microalgal biomass and lipid productivity for commercial scale exploitation of microalgae for biofuel production.
Abstract Title:
Iron Chelator-Mediated Biotransformation of Lignin by Novel Sp., Tolumonas Lignolytica BrL6-1, in Anoxic Conditions
Primary Author Block:
G. M. Chaput, K. M. DeAngelis; Univ. of Massachusetts Amherst, Amherst, MA
Abstract Body:
Climate change is a current crisis that requires reduced reliance on non-renewable fuels, and lignocellulose offers an abundant and undervalued renewable fuel alternative. Lignin is a strong barrier to the conversion of cellulose to biofuels, and lignin valorization is necessary for lignocellulosic biofuels to be market competitive. As the largest natural source of aromatics, lignin is potentially a valuable source of flavors, fragrances, dyes, and biofuels. A mechanistic understanding of anaerobic microbial lignin modification is required for lignin valorization (1,2). Tolumonas lignolytica BrL6-1 is a novel, facultative anaerobic bacterium that was isolated from tropical forest soils on lignin as sole carbon source (3). We hypothesize that when grown anaerobically in the presence of lignin, BrL6-1 produces small molecules that act as both iron chelators and redox agents. These molecules should generate organic free radicals and cause a radical cascade that modifies and depolymerizes lignin. To test this hypothesis, BrL6-1 is grown in lignin-amended media and un-amended media controls. In the presence of lignin, BrL6-1 had a greater biomass and shorter lag phase compared to un-amended conditions. Proteomics showed that during early exponential phase, 18% of the up-regulated proteins (based on 2-fold change or greater) were related to iron transport when lignin was present, and although BrL6-1 encodes genes homologous to lignin degrading enzymes, there was no difference in cytoplasmic abundance compared to controls. Secreted proteins separated by SDS-PAGE exhibited differential banding, with a 20kDa band present in lignin-amended conditions. LC/MS revealed the presence of a hypothetical protein of unknown function with homology to several enzymes in the radical SAM superfamily that are known to produce organic free radicals. Supernatant fractions were tested for chelating ability via Arnow assays, and chelating compounds <10kDa were detected in the lignin-amended supernatant. So far, results support our working model of BrL6-1 lignin modification by small molecules. Future directions include ferrozine assays to test the redox activity of secreted small molecules, experiments to test the effects of Fe(II) bioavailability on BrL6-1 growth, and dialysis experiments analyzed by ICP-MS to determine the binding affinity of iron to lignin and its effect on iron bioavailability. If our hypothesis is correct, this would be the first demonstration of anaerobic microbial free radical generation for lignin modification to our knowledge.
Abstract Title:
Role of Catabolite Repressor Mig1 in Hyper-Cellulolytic Fungus Penicillium Funiculosum Ncim1228

Primary Author Block:
A. Randhawa, O. A. Ogunyewo, D. Iqbal, S. S. Yazdani; Intl. Ctr. for Genetic Engineering and Biotechnology, New Delhi, India

Abstract Body:
Background: Recent bioprospecting to overcome recalcitrance of lignocellulosic biomass identified Penicillium funiculosum NCIM1228 having superior cellulase than presently available commercial formulations1. For its transformation as an industrial workhorse, an obligatory genetic alteration needed was the alleviation of carbon catabolite repression (CCR)2. Mig1/Cre, a transcriptional regulator, is the chief mediator of CCR in fungi. Mig1 perpetuates carbon homeostasis by negatively regulating genes involved in secondary carbon source utilization2. Methods: Genotypic and phenotypic analysis were done to identify and characterize Mig1 homolog from P. funiculosum, PfMig1. Disruption of PfMig1 was achieved by replacing it with null allele Mig188 by Agrobacterium-mediated transformation. Both parent and mutant strains were examined for mycelial growth and glucose uptake in liquid growth medium. Deregulation of cellulolytic enzymes was assessed by measuring transcript levels of major cellulase genes under repressing conditions. Further, increase in cellulase titer was measured by enzyme assays of secretomes of the parent and mutated strain. Results: CCR was maintained in NCIM1228 by a truncated yet functional allele, PfMig1134. Its disruption in NCIM1228 (PfMig188 strain) lead to 30% increased mycelial growth and 1.75-fold better glucose utilization. Microscopy revealed profuse branching pattern in terminal hyphae of PfMig188 strain resulting in small and compact colonies compared to NCIM1228. Transcript levels examined in real-time revealed >10-fold increased expression of two major classes of cellulase, namely, exocellulase and endoglucanase in PfMig188 as compared to NCIM1228, whereas other two major classes, namely, xylanase and b-glucosidase were marginally increased. Similar results were achieved when secretomes of PfMig188 and NCIM1228 were measured for exocellulase, endoglucanase, xylanase and b-glucosidase activities. Further, we found 2-fold increase in synergistic cellulase activity (measured in terms of Filter Paper Units) of PfMig188 secretome compared to NCIM1228. Conclusions: Mig1 disruption in NCIM1228 lead to better growth, enhanced carbon source utilization and profuse branching pattern in terminal hyphae. CCR disrupted strain PfMig188 showed deregulated expression of exocellulase and endoglucanase. Increased expression of exocellulase and endoglucanase in PfMig188 strain resulted in 2-fold increase in total cellulase activity of its secretome.
**Session Number:** 430  
**Session Type:** Poster

**Session Number:** 430  
**Session Type:** Poster

**Session Title:** AES05 - Biofuels and Bioproducts: Making the Most of Microbial Manufacturers

**Session Start Date Time:** 6/10/2018 12:45:00 PM  
**Session End Date Time:** 6/10/2018 2:45:00 PM

**Session Primary Track:** Applied and Environmental Science

**Abstract Control Number:** 5035

**Poster Board Number:** SUNDAY - 806

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**Abstract Title:**
Free Radical Scavenging Activity of Bacterial Pigments

**Primary Author Block:**
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**Abstract Body:**

Synthetic pigments have been extensively used in different industries and these pigments contain toxic substances that may pose threat to human health and in the environment. An alternative source is from natural pigments which can be found from bacteria which may harbor medicinal effects and has higher compatibility with the environment. Thus, this study aims to isolate pigment-producing bacteria and extract their corresponding pigment for evaluation of their antioxidant capability and characterize these pigments. Isolation of bacteria was carried out using spread plate method from agricultural wastes such as corn cobs, rice hull and sugarcane bagasse, following extraction of bacterial pigments by solvent system method. Antioxidant capacity of bacterial pigments was evaluated by their free radical scavenging activity using DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay. Identification was done thru 16S rRNA gene sequencing. The extracted pigments were characterized by maximum absorbance spectrum using the spectrophotometer. Twelve bacterial isolates exhibiting pigmentation were isolated; five from corn cobs, five from rice hulls and two from sugarcane bagasse. Different hues of red (25%), orange (33%) and yellow (42%) colonies were visualized. Bacterial pigments with strongest DPPH activity were Micrococcus luteus (90.51%), Novosphingobium lindanolicasticum (63.19%) and Kocuria indica (59.45%) at 0.20mg/ml pigment concentration. Micrococcus luteus, Novosphingobium lindanolicasticum and Kocuria indica were isolated from sugarcane bagasse, corn cob and rice hull, respectively. The yellow pigment from Micrococcus luteus and the red-orange pigment from Kocuria indica was extracted using methanol while the bright yellow pigment from Novosphingobium lindanolicasticum was extracted using acetone. Spectral analysis showed maximum peak for Micrococcus luteus at 440nm, Novosphingobium lindanolicasticum at 450nm and Kocuria indica at 470nm. Each pigment also showed shoulder peaks. The pigments extracted can be classified as a carotenoid since each pigment exhibit three peaks and these pigments absorb light within the range of 400-500nm. This study demonstrates that pigments extracted from bacteria can be a source of free radical scavengers. The pigments were classified as a carotenoid which is an effective antioxidant.
Isolation and Dev. of Bacillus Megaterium Sr7 For Biofuel Production and Recovery Under Supercritical Co2

Primary Author Block:
A. J. E. Freedman1, J. Boock1, G. Tompsett1, M. T. Timko2, K. L. J. Prather1, J. R. Thompson1; 1Massachusetts Inst. of Technology, Cambridge, MA, 2Worcester Polytechnic Inst., Worcester, MA

Abstract Body:
Supercritical carbon dioxide (scCO2) is an attractive substitute for conventional organic solvents due to its unique transport and thermodynamic properties, its renewability and labile nature, and its high solubility for compounds such as alcohols, ketones and aldehydes. However, biological systems that use scCO2 are mainly limited to in vitro processes due to its strong inhibition of cell viability and growth. To solve this problem, we used a bioprospecting approach to isolate a microbial strain capable of growth under scCO2. Enrichment culture and serial passaging of deep subsurface fluids from the McElmo Dome scCO2 reservoir in biphasic aqueous media with scCO2 headspace enabled the isolation of spore-forming Bacillus megaterium SR7. Sequencing and analysis of the complete 5.51 Mbp genome and physiological characterization revealed the capacity for facultative anaerobic metabolism, including fermentative growth on a diverse range of organic substrates. Supplementation of growth media with l-alanine for induction of spore germination significantly improved growth frequencies and biomass accumulation under scCO2 headspace. Detection of endogenous fermentative compounds in cultures grown under scCO2 represents the first observation of bioproduct generation and accumulation under this condition. To leverage SR7's biocompatibility with scCO2 we sought to genetically modify the strain to generate products that would partition into scCO2. Transformation of SR7 was achieved using a protoplast-based method, permitting identification of promoters for inducible heterologous protein expression in both aerobic and anaerobic conditions. We engineered SR7 to produce isobutanol by a two-enzyme (2-ketoisovalerate decarboxylase (KivD) and alcohol dehydrogenase (Adh)) pathway. A library of Adh proteins was screened to identify enzymes that rapidly convert the isobutyraldehyde intermediate since this compound is expected to highly partition into the scCO2 phase. Combining our recombinant biofuel strain with scCO2 culturing, isobutanol production was observed with co-production of isopentanol, representing the first recombinant bioproducts generated from bacteria grown under scCO2. For cultures that showed high metabolic activity under scCO2, we found almost 50% conversion of the α-ketoacid substrate to biofuel product. Culturing, development and metabolic engineering of B. megaterium strain SR7 represent initial efforts towards enabling exploitation of scCO2 as a sustainable solvent for in vivo bioprocessing.
Abstract Title:
Ciliated Protozoa As Novel Biofuel Organisms
Primary Author Block:
J. L. Black, M. M. Mellen, J. D. Leblond, S. G. Berk, M. B. Farone; Middle Tennessee State Univ., Murfreesboro, TN
Abstract Body:
Background: Although algae have been considered as biofuel feedstocks, there are still shortcomings, such as the requirement for light and the energy input for breaking the cells. For the present study we propose novel organisms as a biofuel feedstock—ciliated protozoa, single-celled organisms that can accumulate lipids similar in type and concentration to those in algae. Methods: Seven strains of the ciliate Tetrahymena were screened for their ability to reproduce rapidly on spent yeast slurry from microbreweries and on water extracts of produce waste from groceries and a packing industry. Ciliates were obtained from the Cornell Tetrahymena Stock Center. Yeast slurries were obtained from two microbreweries in TN. Spent yeast slurries used to culture ciliates were diluted 1:6 to 1:12 with spring water, amended with dextrose (0.5% final concentration), and pH-adjusted to approximately 6.2 prior to sterile-filtering. Produce extracts from lettuce, spinach, and cabbage were prepared by chopping produce in a food processor in a 1:2 ratio (produce to water, by weight), then filtering after 30 min. In one trial, Kudzu leaves were tested. Ciliates were cultured in the brewery and produce wastes at 25°C for 3 days, after which lipids were extracted for selected cultures, and lipid profiles were established. Concentrations of specific lipids per ciliate cell were determined for certain tests. Results: Three of the seven strains grew well in all test media. Numbers of ciliates reached from 5.7x10³ to 7.8x10⁴/ml. Lipid profiles were consistent in all trials for which lipid analyses were done. Among the predominant lipids was linoleic acid, an important precursor for biodiesel. Lipid profiles were similar to those of algae. Conclusions: This work shows that Tetrahymena may be a potential biofuel feedstock, reaching high numbers of cells in only 2-3 days, growing on waste products, and producing lipids used for biodiesel without the need for light.
Abstract Title:
Growth and Bioenergetics Properties of Facultative Chemolithoautotrophic Bacterium Ralstonia Eutropha Upon Organic Waste Materials Utilization

Primary Author Block:
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Abstract Body:
Background: Ralstonia eutropha is a facultative chemolithoautotrophic bacterium able to grow with organic substrates or H2 and CO2 under aerobic conditions. This bacterium is attractive organism with great biotechnological applications. Waste materials such as lignocelluloses containing brewery spent grains hydrolysate (BSGH) and glycerol can be used for bacterial biomass and many valuable compounds, metabolites, polymers and enzymes production. Methods: Growth and bioenergetics properties, such as the FOF1-ATPase activity of R. eutropha H16 during aerobic cultivation on various growth media, pH 7.0, were studied: heterotrophic cultures were grown in mineral salts medium (FN) containing 0.4% w/v fructose alone or supplemented with 0.4% w/v glycerol and BSGH. Bacterial growth was estimated by a spectrophotometer, and biomass yield was calculated by balancing bacterial culture dry weight (CDW); the dilute acid pretreatment method was used to hydrolyze the lignocellulose structure of brewery spent grains. ATPase activity was determined by liberation of Piorg during ATPase reaction. Results: After 24 h of bacterial growth on FN with 0.4% fructose supplementation biomass formation reached up 2.3 g/L CDW. Moreover, bacterial cell mass was stimulated 1.3 fold upon glycerol supplementation. BSGH is typical hemicellulosic hydrolysate which might contain various sugars and formic, acetic acids, etc. Bacteria were able to grow on BSGH with the cell yield of 0.4 g/L CDW. Moreover, pH drop from 7.0 to 6.7 was observed upon growth on FN both with fructose and glycerol utilization, whereas upon growth on BSGH medium pH increased with 0.3 units. The overall and N,N'-dicyclohexylcarbodiimide-sensitive ATPase activity of membrane vesicles were studied grown on FN medium (0.4% fructose). The FOF1-ATPase activity 93±5 nMol Pi (min μg protein)-1 value was observed. Conclusions: R. eutropha can grow well upon inexpensive materials (BSGH and glycerol) utilization having ATPase activity level of 93±5 nMol Pi. Present study in this field is novel: results will be useful in biotechnology for obtaining R. eutropha biomass using different organic wastes.
Abstract Title:
Saccharis: An Automated Pipeline to Streamline Discovery of Carbohydrate Active Enzyme Activities Within Polyspecific Families and De Novo Sequence Datasets

Primary Author Block:
W. Abbott, D. Thomas, D. Jones, A. Ghavidel, N. Alger, D. Inglis; Agriculture and Agri-Food Canada, Lethbridge, AB, Canada

Abstract Body:
Background: Sequence identification of carbohydrate active enzymes (CAZymes) continues to outpace their functional characterization. To direct sequence-based discovery and characterization of new enzyme activities we have developed an automated in silico pipeline entitled: Sequence Analysis and Clustering of CarboHydrate Active enzymes for Rapid Informed prediction of Specificity (SACCHARIS). This pipeline streamlines the selection of uncharacterized sequences for discovery of new CAZyme or CBM specificity from families currently maintained on the CAZy website or within user-defined datasets.

Methods: SACCHARIS extracts entire sequence lists from a designated CAZyme family, en bloc trims multimodular enzymes to their modular boundaries, aligns the trimmed sequences, and displays statistically derived phylogenies with vector graphics suitable for publication. The outputs from this pipeline provide direct and easy-to-interpret insights into new functional space within a CAZyme or CBM family, and entire genomes. Results: SACCHARIS was used to generate a phylogenetic tree of glycoside hydrolase family 43 (GH43) and carbohydrate binding module family 6 (CBM6). These analyses confirmed that large datasets can be organized into sequence clusters of manageable sizes that possess related functions using automated approaches. Seeding characterized trees with unknown sequences (i.e. BdGH43 and CcCBM6) led to the discovery of the first described α-glucanase for GH43 and yeast mannan binding specificity in the literature. Additionally, we have performed a CAZome analysis of an in-house sequenced bacterial genome and performed comparative analysis between B. thetaiotaomicron VPI-5482 and B. thetaiotaomicron 7330, to demonstrate that SACCHARIS can generate “CAZome fingerprints”, which differentiates between the saccharolytic potential of two closely related strains in silico. Conclusions: Establishing sequence-function and sequence-structure relationships in polyspecific CAZyme families are promising approaches for streamlining enzyme discovery. SACCHARIS facilitates this process by embedding CAZyme and CBM family trees generated from biochemically and structurally characterized sequences, with protein sequences that have unknown functions. In addition, these trees can be integrated with user-defined datasets to inform experimental characterization of new CAZymes or CBMs not currently curated, and for researchers to compare differential sequence patterns between entire CAZomes.
Abstract Title:
Ethanol Production from Cassava Starch Using Palm Wine Yeasts Immobilized on Gluten from Wheat

Primary Author Block:
T. M. Adeleye; Federal Univ. of Agriculture Abeokuta, Abeokuta, Nigeria

Abstract Body:
Thirty one yeasts isolated from palm wine obtained from different locations in Abeokuta South West Nigeria, were screened for fermentative abilities. Further screening was carried out in cassava starch liquefied with commercial alpha amylase at 4% (w/v) (pH 6.0, 80°C) and saccharified with glucoamylase at 4% (w/v) (pH 4.5, 60°C). Analyses of volumetric ethanol productivity (Q), carbon dioxide productivity (QCO2) and ethanol tolerance of selected isolates were carried out using standard chemical methods. Yeast isolate with the most desirable traits was selected for further studies and immobilized on gluten beads (1.0cm and 1.5cm) prepared from wheat flour. Yeast isolate designate T01 was selected based on its ethanol tolerance, rate of fermentation and ethanol productivity. Kinetic studies of fermentation of hydrolysed starch by isolate designate T01, showed that residual sugar concentration was directly proportional to viable cell count but inversely related to concentration of ethanol produced. There was no significant difference in ethanol production using yeasts immobilized on 1.0cm bead sizes and free cells. However, yeasts cells of designate, T01 immobilized on gluten pellets of 1.5cm gave a 30% increase in fermentation efficiency and improved ethanol production over free cells. When isolate designate, T01 immobilized on gluten bead (1.5cm) were used in three repeated batch fermentation of cassava starch hydrolysate, there were no significant differences (p>0.05) in all fermentation parameters measured. Results from this study presents shows that cheap agricultural materials such as wheat-gluten is a re-usable and efficient support for immobilization yeast cells in fermenting cassava starch for ethanol production.
Contaminated Sugarcane Must Didn’T Affect Saccharomyces cerevisiae Fermentation

Primary Author Block:
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Abstract Body:
Fermentation process for ethanol production usually don’t operate in aseptic conditions, allowing the proliferation of microbial contamination that can affect Saccharomyces cerevisiae fermentation by decreasing process yield, reducing yeast cell viability and inducing yeast flocculation. Despite of the contaminant microbial diversity frequently reported in fermentation tanks, studies that aimed to understand the relation between S. cerevisiae and contaminant microorganisms usually focus in one microorganism at a time but by doing that, they can misinterpret how this contaminant microbiota works synergistically. Thus, our work aimed to evaluate the effect of microbial-contaminated sugarcane must in fed-batch fermentation with S. cerevisiae recycling. Sugarcane must was collected from a sugarcane mill located at São Paulo state, Brazil. Fermentation recycles with acid treatment were conducted with three initial fermentation cycles using sterilized sugarcane must (121 °C, 20 min) and three final fermentation cycles using non-sterilized sugarcane must. Microbial contamination as well as organic acids, glycerol and ethanol were monitored in fermentation cycles. Non-sterilized sugarcane must presented values of 103 and 108 CFU mL-1 of wild yeast and bacteria contamination, respectively, and increased lactic and acetic acids, glycerol and ethanol concentrations during storage. During fermentation recycles with sterilized and non-sterilized sugarcane must, S. cerevisiae viability and budding rate did not change, and variations observed in ethanol yield between 74.08 to 80.17 % did not seem to be related with must change. Variations were observed in the increase of acetic acid from 0.13 to 0.31 g L-1 and of lactic acid from 0.60 to 1.79 g L-1 in fermentation conducted with non-sterilized must, increase that was not observed in fermentation performed with sterilized must. Also, values of microbial contamination raised from 102 to 103 CFU mL-1 and 104 to 106 CFU mL-1 for wild yeast and bacteria, respectively, in fermentations conducted with non-sterilized must. Besides the increase in microbial contamination and lactic and acetic acids, ethanol yield, yeast budding and viability were not affected by the microbial contamination present in non-sterilized sugarcane must. As microbial contaminants are known to cause problem in fermentation process, future works should focus on understanding what truly relies on the contamination that causes these problems and what are the triggers or the events that are related with harmful contamination.
Abstract Title:
Improving Microbial Production of Isobutene
Primary Author Block:
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Abstract Body:
In the rapidly expanding field of biotechnology, biofuels still face serious hurdles to become economically viable. To overcome these hurdles, a multidimensional engineering strategy is needed. Improvements must be made not only in production efficiency, but in the range of feedstocks used for chemical bio-production. Isobutene is a short chain gaseous hydrocarbon used to make fuel additives, detergents and butyl-rubbers with a global market value of roughly 19 Billion USD/year. E. coli MG1655 was engineered to make isobutene through expression of two enzymes, M3K (P. torridus) & MVD (S. cerevisiae). M3K and MVD were inserted into an intergenic region of the central chromosome of E. coli for constitutive expression, using CRISPR/Cas counterselection in tandem with λ-red recombinases. The two enzymes were integrated together due to research suggesting cells expressing both enzymes would produce more isobutene than cells expressing either singly. Growth curves demonstrate that insertion of M3K and MVD do not detrimentally affect growth rates. An airtight pressure monitoring system was developed to maintain integrity and accuracy of gaseous measurements. Using this system production of isobutene was confirmed via GC-MS analysis with 132ppm after 24hrs. The effects of tolerance engineering on isobutene production will be assessed. Cultures will be stressed with hydrocarbon solvents, and the transcriptomic changes will be assessed to determine viable genes for regulation manipulation to increase isobutene titers. Transcriptome data will be gathered from 8 biological replicates, mRNA isolated and sequenced using Illumina MiSeq, using a V3 600 cycle kit. This data will be cleaned using Trimmomatic, and annotated using DIAMOND. Stress samples will be screened against normal control to determine changes in expression of stress response and transporter proteins. The genes that conveyed increased tolerance when overexpressed will be selectively overexpressed in our engineered strain to increase tolerance to isobutene production. Finally, ability of this production strain to use sewage waste as a feedstock will be assessed. This will allow us to determine the efficacy of this engineered strain as a waste remediator. All isobutene production will be analyzed from culture headspace using GC-MS. This will demonstrate the value of biofuel production strains not only as chemical producers, but waste remediators as well.
Abstract Title:
Molecular Breeding of Surface-Engineered Yeast for Efficient Biorefinery of Alginate and Mannitol

Primary Author Block:
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Abstract Body:
Brown macroalgae are sustainable and promising biomass resources because they are abundant in ocean ecosystems and does not contain lignin peculiar to terrestrial plants. However, conversion efficiency of macroalgae to ethanol is low with the technique based on existing technology because the polysaccharide composition is different from that of terrestrial plants. Among the main polysaccharide components such as alginate, mannitol, and laminarin, alginate accounts for about 20-40% of the dry weight of brown macroalgae. However, the utilization mechanism by marine bacteria is not well known and its effective utilization has not progressed. In this study, molecular breeding of novel yeast strain that can directly utilize alginate and mannitol from brown macroalgae was attempted by cell surface engineering (1) and synthetic biology/metabolic engineering. We focused on marine bacteria with high seaweed assimilation capability, and identified enzymes involved in seaweed assimilation by omics analysis (2). Based on this information, endolytic and exolytic alginate lyases for degradation of alginate into monosaccharide, DEH (4-deoxy-L-erythro-5-hexoseulose uronic acid), were co-displayed on the cell surface of yeast Saccharomyces cerevisiae (3). In addition, the genes encoding DEH transporter and components of the DEH metabolic pathway additionally were overexpressed in the yeast co-displaying alginate lyases. Furthermore, to maintain cellular redox balance, the mannitol-metabolizing capacity of the constructed yeast was enhanced by prolonged culturing in a medium containing mannitol as the sole carbon source. The resulting yeast strain AM1 enabled co-metabolism of alginate and mannitol, and directly produced 8.8 g/L of ethanol from 60 g/L of sugar mixture containing of alginate and mannitol at a ratio of 1:2 (4). Therefore, this refinery system using the AM1 strain should contribute to the efficient utilization of brown macroalgae for ethanol production. (1) K. Kuroda and M. Ueda (2013) Biomolecules, 3, 632-650 (2) T. Takagi et al. (2016) Mar. Biotechnol., 18, 15-23 (3) T. Takagi et al. (2016) Appl. Microbiol. Biotechnol., 100, 1723-1732 (4) T. Takagi et al. (2017) Appl. Microbiol. Biotechnol., 101, 6627-6636
Abstract Title:
Deciphering Niche Differentiation among Phylotypes in A Thermophilic Digester

Primary Author Block:
V. Lhilhi Noundou1, S. Malkaram1, E. Chavarria-Palma1, N. Montenegro-Garcia1, i. Ugwuanyi1, T. Espinosa-Solares2, D. Huber1; 1West Virginia State Univ., Institute, WV, 2Univ. Autonoma Chapingo, Chapingo, Mexico

Abstract Body:
Anaerobic digestion (AD) is one of the most successful industrial-scale, mixed-culture microbial processes. In AD, crude organic matter is decomposed in a complex food web that is traditionally described as four trophic levels: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. One strength of the AD process is the adaptability of the food web with regard to different substrates and environmental conditions within the reactor. However, the efficiency of AD processes is variable and reactors can fail for no apparent reason. Little is known about the environmental tolerances, niche requirements and position in the food web for most of the bacterial phylotypes. Using correlation analysis, we investigated the relationships between environmental conditions, intermediate metabolites, and phylotype abundance within a thermophilic AD microbiome in order to begin deciphering niche specialization. Five replicate 10 liter reactors were operated for more than 400 days with a very low C/N ratio substrate (poultry litter). A second feedstock (crude glycerol) was periodically added to the reactors which provided substrate for new fermentation pathways and short term increases in chemical oxygen demand (COD). Anaerobic food web linkages were evaluated by monitoring several key metabolites (fatty acids, methane, ammonia, pH) with standard HACH tests and gas chromatography. Microbial diversity was measured at five time points using Illumina sequencing and Earth Microbiome Project protocols to target 16S rRNA gene diversity. Nearly 8 million paired-end reads were obtained. The most abundant bacterial phyla were Firmicutes, Proteobacteria, Thermotogae and Bacteroidetes. These phyla showed significantly different patterns of correlation for these variables. For example, Firmicutes and Thermotogae abundances were not correlated with ammonia, while Proteobacteria was positively and Bacteroidetes was negatively correlated. Subdividing the Firmicutes by order showed further differentiation. Clostridiales was positively correlated with total volatile acids while SHA-98 was negatively correlated. This analysis is helping to reveal niche differentiation and trophic relationships for unknown digester phylotypes.
Abstract Title:
A Novel Trifunctional, Family Gh10 Enzyme from Acidothermus Cellulolyticus 11b, Exhibiting Endo-Xylanase, Arabinofuranosidase and Acetyl Xylan Esterase Activities

Primary Author Block:
S. Shahid, R. Tajwar, M. W. Akhtar; Univ. of the Punjab, Lahore, Pakistan

Abstract Body:
Degradation of hemicellulosic fraction of plant biomass is essential for the pre-treatment of poultry cereals, bio-bleaching of wood pulp and biofuel industry. Hemicellulose is made up of xylan backbone that is degraded the xylanases. However, xylan backbone is decorated with various side chains including arabinose and acetic acid that hinder the activity of endo-xylanases. This study reports a multifunctional endo-xylanase (Xyn10B) from Acidothermus cellulolyticus 11B that not only cleaves the xylan backbone but also facilitates the hydrolysis of arabinose and acetyl side chains of various hemicellulosic substrates. Xyn10B was cloned and expressed in Escherichia coli and purified to homogeneity by binding to regenerated amorphous cellulose. It had higher binding on Avicel as compared to insoluble xylan due to the presence of cellulose-binding domains, CBM3 and CBM2. This enzyme was optimally active at 70 °C and pH 6.0. It was stable up to 70 °C while the CD spectroscopy analysis showed thermal unfolding at 80 °C. Xyn10B was found to be a trifunctional enzyme having endo-xylanase, arabinofuranosidase and acetyl xylan esterase activities. Its activities against beechwood xylan, p-Nitrophenyl arabinofuranoside and p-Nitrophenyl acetate were found to be 126,480, 10,350 and 17,250 U μmol⁻¹, respectively. Xyn10B was highly active producing xylobiose and xylose as the major end products, as well as debranching the substrates by removing arabinose and acetyl side chains indicated in the HPLC chromatograms. Due to its specific characteristics, this enzyme seems to be of importance for industrial applications that involve degradation of plant biomass.
Abstract Title:
Effect of Growth Hormones on Three Native Algal Strains for Biofuel Production
Primary Author Block:
B. H. Edwards, Ill1, R. Rathore1, R. Jaswal1, A. Chauhan1, J. Wen2, M. Zhou2, Z. Syed2; 1Florida Agriculture and Mechanical Univ., Tallahassee, FL, 2Lawton Chiles High Sch., Tallahassee, FL
Abstract Body:
Microalgae holds an immense potential for production of biodiesel and other value-added products. When growth is coupled to the nutrients present in wastewater, this becomes an environmentally sustainable technology. In this study, we compared lipid productivity of 10 previously isolated strains that are native to Tallahassee’s wastewater holding tank. A preliminary taxonomic analysis of the 10 strains revealed that these are a mix of microalgae, cyanobacteria, bacteria and fungi. We are in the process of further evaluating this diversity using metagenomics because there is a strong possibility of symbiotic association between the above stated microorganisms for both wastewater remediation and production of lipids. When a comparison was drawn between the abilities of these strains to produce lipids, we found that strains #3, #4, #9 and #11 were the most efficient when grown in synthetic BG11 media. Our next goal was to compare the strain’s growth efficiencies in influent and effluent wastewater. However, excessive nutrients in the wastewater is known to inhibit efficient lipid productivity. Towards this end, amending the growth with hormones have shown to promote lipids and biomass productivity of microalgae. Therefore, we supplemented the algal strains, in particular, strains #3, #4, #9 and a mix of these as a consortium, with natural abscisic acid (ABA), synthetic 2,4-dichlorophenoxyacetic acid (2,4-D) and 1-naphthaleneacetic acid (NAA) at 0.5, 1.0 and 1.5 mg/L in BG11 synthetic media as well as both, influent and effluent wastewater. We ran the treatments in triplicates with appropriate controls and collected samples every two days, over a course of 16 days, and analyzed lipid productivity by using 2 mg (dry weight biomass) by the sulfo-phospho-vanillin (SPV) method. Overall, our finding indicates that algal strain #4 at 0.5 mg/L 2,4-D hormone concentration showed maximum lipid production relative to other hormones and strains tested. Additionally, the consortial strains also showed enhanced lipid production at the same concentration of 2,4-D hormone, suggesting that hormonal treatments may enhance production of sustainable microalgal biofuels using wastewater as a growth media.
Abstract Title:
Impact of the Inoculum and Starting Parameter of A Fermentor on Digesting Microbial Communities and their Biogas Production

Primary Author Block:
P. H. R. Mallinger, M. Melendrez-Vallard, R. C. Fink; St. Cloud State Univ., St. Cloud, MN

Abstract Body:
In recent years, biogas has been considered as a source of environmental friendly energy. Biogas output in fermentors is determined by a diverse microbial community, with methanogens as the main methane producers. These are usually part of the microbial community of the initial inoculum and their establishment in the fermentor community depends on environmental conditions and size of the initial inoculum. In the work we present, we manipulated organic waste composition and amount of initial inoculum to test the impact of these parameters on anaerobiosis onset and biogas output. We conducted two separate experiments (E1 and E2) in a bench top fermentor system using food waste sourced from St. Cloud State and a manure inoculum from a local farm. We used a ratio 1:10 of manure vs. feedstock in E1 and a 1:5 ratio in E2. The slurry and digestates chemical compositions were determined by soil chemical tests (K, NO₃, N, P, Al, NH₃). Microbial community was characterized throughout the experiments using 16S next generation sequencing. The two experiments were carried over the same time period, 60h. fermentor oxygen content was tested using anaerobic test strips. Sampling of digestate and gas production was performed in triplicates, at fixed intervals, and on a logarithmic scale. Chemical composition, pH, and microbial community of samples were characterized at every sampling time. In E2 the initial feedstock was rich in fat and had a higher ammonia content (2 ppm) than in E1 (no ammonia content and low fats). Onset of anaerobiosis in the fermentor occurred earlier in E2 than in 1 (24 and 60 h respectively), possibly for the more abundant microbial community due to the lower ratio of manure vs. feedstock. In E2, biogas production started earlier than in E1 (1 and 4 h respectively), peaked early, but dropped quickly to zero (12 h from the peak). This resulted in a 10-fold lower total biogas yield in E2 than in E1. This was mirrored by changes of the overall diversity of the microbial communities with a sharp decrease in diversity in E2 after the gas production stopped. NH3 in E2 increased to more than 10 ppm. This change might have negatively impacted the microbial community thus affecting biogas production. Also, the high fat content in E2 could have been a factor. Recent studies indicated oily fats can impact the anaerobic fermentation by inhibiting methanogens metabolism and causing digester foaming, which we observed. Ongoing analysis will be conducted to reveal specific metagenomics and metabolic cycling analysis of microbial community composition.
Engineering the Cellulolytic Extreme Thermophile Caldicellulosiruptor Bescii For the Reduction of Carboxylic Acids to Alcohols

Primary Author Block:
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Abstract Body:
Caldicellulosiruptor bescii is the most thermophilic cellulolytic organism currently known, having an optimal growth temperature of 78°C. This anaerobic bacterium simultaneously ferments pentose and hexose sugars to acetate, lactate, hydrogen, and carbon dioxide. With its ability to grow on completely untreated plant biomass and because it has an established genetic system, C. bescii is a promising microbial platform for lignocellulose conversion to bio-based fuels and chemicals. In this study, we report the characterization of two C. bescii strains that heterologously express the AOR-Adh pathway for the reduction of carboxylic acids to alcohols. The heterologous expression of the aldehyde ferredoxin oxidoreductase (aor) from Pyrococcus furiosus and a primary alcohol dehydrogenase (adhA) from Thermoanaerobacter sp. X514 allows C. bescii to recycle the reduced ferredoxin and NADH generated in glycolysis in the reduction of acetate to acetaldehyde and ultimately to ethanol. Deletion of the native C. bescii lactate dehydrogenase (ldh) in a strain expressing the AOR-Adh pathway further increased ethanol production. In these engineered strains, we demonstrate production of up to 4 mM ethanol, as well as the reduction of exogenously-supplied organic acids into their corresponding alcohols, specifically isobutanol and hexanol. This work has provided proof of principle that the AOR-Adh pathway can be harnessed for both the production of ethanol and the conversion of other organic acids to alcohols by the oxidation of sugars derived from lignocellulosic biomass.
Abstract Title:
Aerobic and Anaerobic Cellulose Utilization by Paenibacillus Sp. Caa11 and Enhancement of Cellulosic Ethanol and Organic Acids Production by Expressing A Single Heterologous Endoglucanase

Primary Author Block:
Y. Um, E. Kim, S-M. Lee; Korea Inst. of Sci. and Technology, Seoul, Korea, Republic of

Abstract Body:
Background: For cost-effective lignocellulosic biofuel/chemical production, consolidated bioprocessing (CBP)-enabling microorganisms utilizing cellulose as well as producing biofuel/chemical are required.

Methods: In this study, a novel strain of Paenibacillus sp. CAA11 isolated from foreshore sediments was investigated as a potential facultative CBP-enabling microorganism. Growth characterization of Paenibacillus sp. CAA11 was performed with respect to initial pH, temperature, sugars, and cellulose. Expression of heterologous endoglucanase was carried out to enhance the cellulolytic ability of Paenibacillus sp. CAA11 by applying Bacillus genetic tools. The effect of the enhanced cellulolytic ability on biofuel/chemical production was also evaluated under both aerobic and strict anaerobic conditions.

Results: A novel strain Paenibacillus sp. CAA11 isolated from sediment was found to be not only as a cellulose degrader under both aerobic and strict anaerobic conditions but also as a producer of cellulosic biofuel/chemicals. Paenibacillus sp. CAA11 secreted cellulolytic enzymes by its own secretion system and produced ethanol as well as short-chain organic acids (formic acid, acetic acid, lactic acid) from cellulose. Cellulolytic activity of the strain was significantly enhanced by expressing a heterologous endoglucanase 168Cel5 from Bacillus subtilis under both aerobic and anaerobic conditions. The strain harboring the 168Cel5 gene revealed 2-fold bigger halo zone on Congo-red plate and 1.75-fold more aerobic cellulose utilization in liquid medium compared with the negative control. Notably, under anaerobic conditions, the recombinant strain expressing 168Cel5 consumed 1.83-fold more cellulose (5.10 g/L) and produced 5-fold more ethanol (0.65 g/L) along with 5-fold more total acids (1.6 g/L) compared with the control, resulting 2.73-fold higher yields. Conclusions: This result demonstrates the potential of Paenibacillus sp. CAA11 as a suitable aerobic and anaerobic CBP-enabling microbe with cellulolytic production of ethanol and short-chain organic acids.
Abstract Title:
Ligninolytic Potential of Environmental Klebsiella Spp. Strains Isolated from Soil and Freshwater

Primary Author Block:
T. Bruce1, A. Melo-Nascimento2; 1San Diego State Univ., San Diego, CA, 2Univ.e Federal da Bahia, Salvador, Brazil

Abstract Body:
Background: Microbial enzymes capable of degrading organic material can be useful in biofuel production by replacing or decreasing the use of thermochemical processes to pretreat lignocellulosic biomass. The need for high-yield enzymes to overcome the biochemical recalcitrance of lignin has motivated the bioprospecting of ligninolytic microorganisms in the environment. Methods: Bacterial strains were isolated using lignin as the unique carbon source in the media. Taxonomic assignment of 16S rRNA gene sequences identified the isolates as belonging to the genus Klebsiella. The three fastest-growing strains reached the stationary phase of growth in approximately 24 h. Ligninolytic potential was evaluated by using solid and liquid minimal medium containing dyes that are structurally similar to lignin fragments (toluidine blue, methylene blue, malachite green, and Congo red). Results: On solid medium, enzymatic indices ranged from 0 to 1.25, with the highest values for isolates P3TM.1 and FP10-5.23 in the presence of toluidine blue. In liquid medium, higher levels of dye decolorization by 24 h were obtained for methylene blue, reaching 98% decolorization at 48 h for P3TM.1. Conclusions: Our results suggest Klebsiella spp. associated with fresh water and soil play important roles in the cycling of recalcitrant molecules in the Caatinga biome and represent a potential source of lignin-degrading enzymes with biotechnological applications.
Abstract Title:
Fuel Ethanol Production from Thermotolerant Starch and Xylose Fermenting Microorganisms Isolated from the Natural Fermented Sources of Bangladesh

Primary Author Block:
A. Talukder, R. Mia, A. Siddiqa, J. Ferdous Tuli, N. Barman; Jahangirnagar Univ., Dhaka, Bangladesh

Abstract Body:
Background: Energy crisis and environmental pollution are the two major global problems that become a threat to our life. A solution of these problems [G1] demands the use of a renewable and clean source of energy. In this context, bioethanol is both eco-friendly and renewable source of energy. There are several potential benefits of thermotolerant microbes for using in the production of industrial alcohol. The solubility of oxygen and other gases in the fermentation broth decreases with increasing temperature. Therefore, the energy required to maintain proper agitation of the growth media is reduced. The metabolic activity of microbes and frictional effects of agitation serves to generate large amounts of heat. Thus, additional energy to maintain the vessels at the desired temperature, as well as the cooling requirements after sterilization is reduced. Methods: The optimum fermentation conditions were as follows: Yeast Extract Peptone medium (YP) containing Xylose and Starch, 10% with pH 6.5; Inoculum size, 1.0%; Temperature, 37 °C; Shaking condition, 100 rpm and Fermentation time, 72 h for xylose and 120 h for starch. Bioethanol content was measured by our developed spectrophotometric method for any unknown sample using a solvent tri-n-butyl phosphate [TBP, non-alcoholic solvent, density = 0.975 to 0.976, solubility in water = 0.028% (w/v)]. Our established method showed similar results performed by relatively expensive Gas Chromatography (GC) method. Results: Based on thermotolerant abilities at 42°C, we have screened four thermotolerent microbes using xylose or starch as sole carbon sources from natural fermented sources. Bioethanol content and starch hydrolysis abilities of these strains were also performed. Among the four strains, P-5 showed the highest gluco-amylase activity (2.40 Unit/ml/min) as well as highest ethanol production rate (5.5 and 4.0 % (v/v) from xylose and starch, respectively) under the conditions we have employed here. All four strains could tolerate ethanol well up to 10% at various tested temperatures (25-42°C). Finally, DNA sequencing information revealed that the strains P-5 and P-1 were identified as Pichia kudriavzevii and Mxm-1 and M-3 were Candida tropicalis and Bacillus licheniformis, respectively. Conclusions: We have concluded that the strain P-5 is suitable candidate for laboratory scale fuel ethanol production in a fermenter (3-Litres) from inexpensive biomass like potato starch in Bangladesh, which production process have to be optimized in future.
Abstract Title:
Enhanced Thermotrophic Biomethanation of Rice Straw by Supplementation of Startup Inoculum with Thermophilic Methanogens: Insights from Transcriptome Analysis

Primary Author Block:
S. D. Pore, S. S. Dagar, P. K. Dhakephalkar; MACS Agharkar Res. Inst., Pune, India

Abstract Body:
Biomethanation can be considered as relatively greener and more efficient alternative to combustion or gasification for energy recovery from agricultural wastes such as rice straw. Thermotrophic biomethanation of rice straw (without pretreatment) was attempted as an environment friendly process for the enhanced recovery of energy in the form of biomethane. The startup inoculum (cattle dung) was supplemented with three different strains of thermophilic methanogen Methanothermobacter thermoautotrophicus. Anaerobic digestion of rice straw at 7.5 \% (w/v) loading rate at 55 °C in continuous mode with 10 days hydraulic retention time (HRT) resulted in methane yield of 375 L/kg volatile solids (VS). The obtained methane yield at 10 days HRT is one of the highest for the biomethanation of rice straw in continuous mode. Interestingly, this process resulted in enhanced methane (upto 75\%) and low CO2 content, low accumulation of volatile fatty acids, and faster degradation of different lignocellulosic components. The transcriptome analysis indicated the expression of genes encoding cellulolytic enzymes such as endo-1,4-β-glucanase and β-glucosidase, xylanolytic enzymes such as 1,4-β-xylosidase, xylose isomerase, etc., and key lignin degrading enzyme caffeyl-CoA O-methyltransferase. Metabolic pathway analysis revealed that the major pathway for carbohydrate utilization was glycolysis and pentose phosphate pathway, followed by TCA cycle with CO2, acetate and other volatile fatty acids (VFA) being the major metabolites. The acetate production was achieved via glycolysis and pyruvate metabolism, and not by reductive TCA. Butyrate to acetate transformation was achieved via conversion of butanoyl-CoA to acetyl-CoA. The genes responsible for hydrogenotrophic methanogenesis found dominant over aceticlastic methanogenesis. The faster hydrogenotrophic methanogenesis ensured low accumulation of hydrogen, leading to improved syntrophic fatty acid degradation. Thermotrophic biomethanation of rice straw without thermochemical pretreatment makes this process a viable alternative for sustainable energy recovery from renewable agricultural wastes.
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Session Type: Poster
Session Title: AES05 - Biofuels and Bioproducts: Making the Most of Microbial Manufacturers
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/10/2018 2:45:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 6243
Poster Board Number: SUNDAY - 827

Abstract Title:
Fermentable Sugar Production from Grassland Biomass

Primary Author Block:
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Abstract Body:
Application of lignocellulosic biomass in the production of second generation biofuel has been well recognized over a while. However, many of the technologies and recommendations rely on the use of specific biomass types, e.g., straw, softwood or hardwood, and are too expensive to be transferred outside the lab. Natural and semi-natural grasslands cover around 75 million ha of EU territory. Together, they make up about 39% of the European utilized agricultural area and are mostly used for animal grazing, composting or heating. High transportation costs, low yields (1-12 t dry matter/ha per year), and large species biodiversity are only some of the reasons why grassland biomass is regarded as inferior. The aim of this study was to evaluate the potential of various grassland biotopes from temporary climate with a simple conversion technology employing enzymes extracted from various fungi. Within this study samples from 6120, 6210, 6270, 6410, 6450, 6510 (Natura 2000 codes) and planted grass were analysed with a simple conversion technology which involved mechanical milling (fractions below 0.5 cm) and 5 min boiling (1 atm) as pre-treatment and 24 h enzymatic hydrolysis with laboratory made enzymes from white rot fungi. Viscozyme (SigmaAldrich) was used as a reference enzyme. The results showed that higher fermentable sugar yields (reducing sugar concentration, g/g substrate) can be obtained from biotopes that are rich with dicotyledonous plant species, e.g., No. 6210. Similar trend in lower production yields from monocotyledonous plants with the same pre-treatment/hydrolysis technique has been already described and can be linked with the recalcitrance of the biomass. Assessment of harvesting time showed that samples collected in June presented the highest sugar yields with the tendency to decrease with the increase of vegetation period (Figure). Thus, the study allowed to conclude that enzymatic hydrolysis combined with simple mechanical milling and boiling is suitable for fermentable sugar extraction from all grassland biotopes and fermentable sugar concentration is influenced by the seasonality and proportion of monocotyledonous and dicotyledonous plants within in the sample.
Abstract Title:
Microbial Community in Biowaste-Fed Biodigesters and Exposure Effect of Biogas on Liver Biomarkers of Wistar Rats

Primary Author Block:
S. B. Akinde1, O. P. Olaniyan1, O. O. Adewale1, O. O. Oyedara2, J. O. Olaitan1; 1Osun State Univ., Osogbo, Nigeria, 2Inst. Politécnico Natl., Reynosa, Tamaulipas, Mexico

Abstract Body:
Background: Biogas production from organic wastes is a promising energy generation and biowaste handling alternative, especially in the developing countries. The study examined the bacterial and fungal distribution in slurries from biodigesters made from cheap local materials as well as the exposure effect of the biogas produced on the liver biomarker of Wistar rats. Methods: Three 20-litre capacity plastic prototype biodigesters were constructed and fed with kitchen waste, cassava peel and cow dung in the ratio 1:2 of biowaste to water respectively. Temperature, pH and microbial analysis of slurries from the batch-operated experiment (before and after biogas production) was carried out using standard protocols. Hepatotoxicity biomarkers of Wistar rats post 7-day biogas exposure under a closed system at various daily exposure time was measured with biomarker detection kits. Results: The ambient and slurry temperatures were 23 - 26°C and 25 - 30°C respectively with slurry pH at near neutrality range of 6.9 - 7.3. There was an even distribution of aerobic bacteria and fungi in the slurries (before and after) with log10 values of 4.84 ± 0.25 - 8.54 ± 0.45 and 2.61 ± 0.23 - 7.48 ± 0.39 respectively. Both associated bacterial and fungal genera exhibited cellulolytic activity with zone diameter of 21 - 35 mm. Presence of Enterobacter sp., Proteus sp., Serratia sp. and other potential pathogens with biofilm-forming potentials (moderate/ strong) in post-production slurries is an indication of possible biosafety issues. No significant effect (p > 0.05) of biogas on the alkaline phosphate (ALP), alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), albumin and total protein levels in Wistar rats. Conclusions: The study outcome adds significant knowledge on the microbial diversity of biowaste-fed biodigesters and the biogas effect on liver toxicity biomarkers of Wistar rats. Management of biosafety issues associated with biogas generation from organic wastes is suggested.
Abstract Title:
Batch Fermentation Characteristics and Hydrogen Production of Escherichia coli Wild Type and Hydrogenase Mutants Using Xylose As Feedstock

Primary Author Block:
A. Poladyan, R. Hayrapetyan, A. Trchounian; Yerevan State Univ., Yerevan, Armenia

Abstract Body:
Background: Xylose is one of the most abundant sugars derived from the breakdown of lignocellulosic biomass. Escherichia coli can uptake and utilize many sugars to form biomass and to produce H2 from formate via formate hydrogen lyase (FHL) during E. coli xylose or glucose fermentation. FHL consists of formate dehydrogenase H (FDH) and membrane-associated [Ni-Fe]-hydrogenase (Hyd) enzymes.

Methods: Bacterial biomass yield was calculated by balancing bacterial culture dry weight (CDW); Redox potential (ORP) changes and H2 production were studied with two redox Pt (sensitive to H2) and Ti-Si electrodes: simultaneously using these electrodes upon bacterial growth provides information both on general redox processes and H2 production yield. Results: In the present study (0.05% to 1%) xylose utilization was investigated by E. coli BW25113 wild type parental strain (PS) and ΔhyaB, ΔhyBc, ΔhycE, ΔhyfG mutants with deletions of genes for key subunits of Hyd-1 to Hyd-4 in minimal salts (MSM) and peptone medium (PM), pH 5.5 and 7.5. Some data were compared with that of glucose fermentation. Compared with 0.1%, the biomass yield was enhanced 2 fold upon 1% xylose fermentation (0.60±0.04 CDW, g/L). Upon 0.05% xylose fermentation H2 production was observed at 4th and 5th h log growth phase with the yield of 0.8 mmol/L upon glucose and xylose fermentation, respectively, pH 7.5. H2 production was absent in ΔhycE and ΔhyfG mutants, with defects in Hyd-3 and Hyd-4 at all conditions used. H2 production was stimulated during growth of ΔhybC upon 0.05% xylose fermentation, but upon 1% xylose the same stimulating effect was observed both in ΔhyaB and ΔhybC mutants. At pH 5.5 biomass formation is reduced ~2-fold upon 0.05 and 1% xylose utilization, particularly with the mutations in Hyd-3 and Hyd-4. In contrast to pH 7.5, during E. coli PS growth at pH 5.5, H2 was produced at 2 h early, at the 3th h of bacterial growth with the yield of 0.75±0.05 mmol/ L. E. coli PS and Hyd-mutants growth was reduced β3 fold in MSM at all pHs. In MSM H2 formation was delayed, but observed at 3 h early at pH 5.5 than at pH 7.5. Conclusions: Obtained data indicate that during E. coli xylose fermentation Hyd-3 and Hyd-4 are important for both bacterial growth and H2 production at all conditions used. Moreover, high concentrations of xylose might stimulate Hyd-1 enzyme activity during bacterial growth at pH 7.5. At acidic pH H2 production is observed at the early beginning of bacterial log growth phase. These results are of significance to develop H2 production biotechnology using xylose as a feedstock.
Partial Characterization and Cloning of Protease Gene from Bacillus

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Abstract Body:
The present research focuses on amplification of protease gene from Bacillus strain which was later assessed for maximal enzyme activity, as they are the dominant bacterial workhorses in microbial fermentation. A putative Bacillus strain was isolated from soil, inoculated into protease production media and optimized with appropriate pH and temperature parameters for the maximal enzyme activity. Genomic DNA was isolated from the strain and the fragment was amplified by PCR using gene-specific primers for protease. The fragment was then ligated into a T/A cloning vector and transformed into calcium chloride-treated competent Escherichia coli DH5α cells. The presence of the gene was confirmed in the isolated plasmid. A specific amplification of 1.1 kB fragment was observed following PCR. The amplified product includes the coding sequence and a signal peptide sequence of the protease gene. After cloning with T/A cloning vector pTZ57R/T followed by transformation into E. coli DH5α competent cells, the recombinant plasmid was selected using blue-white selection. Plasmid DNA was sequentially isolated from the recombinant strains, were confirmed in the presence of the gene of interest using PCR and further quantified by Lowry protein assay for maximal protease activity. The optimum pH was found to be 10.1 with an activity of 21.566 IU/ml, and the optimum temperature was found to be 60°C giving an activity of 38.708 IU/ml. Amplification of protease gene isolated from Bacillus strain and optimization of pH and temperature conditions for the assessment of subtilisin Carlsberg produced by it. Subtilisin which is protein engineered can be used in commercial products such as stain cutter, dishwashing detergents, cosmetics and food processing, and contact lens cleaner.
Outstanding Abstract Award: Discovery of Metabolic Pathways Involved in Lipid Production from Lignin-Derived Phenolics in A Non-Model Oleaginous Yeast

Primary Author Block:
A. Yaguchi, M. Spagnuolo, M. Blenner; Clemson Univ., Clemson, SC

Abstract Body:
Interest in lignocellulosic biomass as a feedstock has grown, as cellulosic biomass is rich in C6 and C5 sugars, such as glucose and xylose. The lignin is typically removed, as its high aromatic content is too toxic for most microorganisms. We have found Cutaneotrichosporon oleaginosus, a non-model oleaginous yeast, tolerates and metabolizes lignin-derived phenolics as a sole carbon source while accumulating over 69% of its biomass as lipids. Our studies show C. oleaginosus is able to fully metabolize phenol, 4-hydroxybenzoic acid (pHBA), and resorcinol as sole carbon sources, as well as in co-utilization with either glucose or xylose. At low carbon concentrations, cells growth on pHBA and resorcinol was as robust as in glucose media. Phenol caused an extended lag phase due to toxicity, but cells were able to achieve comparable biomass as cells in other carbon sources. We explored different feeding strategies to overcome aromatic toxicity and increased lipid accumulation to over 69% of biomass by weight. Aromatic metabolism is well-characterized across many organisms; however, there are many different mechanisms utilized between species. Initial BLAST analysis indicated the C. oleaginosus uses the ortho-cleavage pathway; however, conflicting qPCR data suggested alternate enzymes or mechanisms. RNAseq data enabled analysis of all putative aromatic degradation genes, and revealed other participating genes that were missed by initial BLAST analyses. Using transcriptomic analysis, we have elucidated pathways for aromatic metabolism in C. oleaginosus, and are in the process of confirming it with metabolomics data. In addition, we are developing genetic engineering tools to create novel fatty acids from aromatic compounds. In summary, our work demonstrates the potential for this yeast to convert all components of lignocellulosic biomass into value-added products.
Session Number: 431
Session Type: Poster
Session Number: 431
Session Type: Poster
Session Title: AES06 - Bioremediation, Biodegradation, Biofouling, and Biocorrosion II
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/10/2018 2:45:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 7183
Poster Board Number: SUNDAY - 832

Abstract Title:
Evaluation of Different Methods for Characterizing the Microbiome in Aged Oil Spills and Microbial Relationships with Enhanced Magnetic Susceptibility in Petroleum-Contaminated Sediments

Primary Author Block:
C. Beaver1, E. Atekwana2, D. Ntarlagiannis3, L. Slater3, S. Rossbach1; 1Western Michigan Univ., Kalamazoo, MI, 2Univ. of Delaware, Newark, DE, 3Rutgers-Newark, Newark, NJ

Abstract Body:
Background: Oil spills are a recurring problem in our society, and the removal of spilled hydrocarbons depends on the metabolic activity of microbes in the environment. However, the monitoring of bioremediation by geochemical and microbiological methods can be costly and difficult to execute, especially when the biodegradation is occurring at a great depth in an underground aquifer. Newer geophysical methods that measure changes in magnetic susceptibility are less expensive and may be an easier way to track bioremediation in hydrocarbon-contaminated sediments. Where bioremediation is taking place, magnetic susceptibility increases through the activities of iron-reducing bacteria, thus making it possible to detect when these microorganisms are active. Methods: Cores were obtained over a period of five years (2011-2015) from an aged oil spill site in Bemidji, Minnesota. Magnetic susceptibility was measured in the cores. Total DNA was isolated by using two DNA isolation methods, one using the PowerSoil Kit and the other one the Zhou soil DNA isolation method testing their effectiveness. In addition, 16S rRNA gene MiSeq high throughput sequencing was used to identify the petroleum-degrading populations with QIIME or mothur, evaluating both analytical methods. Results: Magnetic susceptibility was elevated in the saturated and vadose zones in the core collected in the free-phase petroleum plume. Areas of high magnetic susceptibility in the free phase plume contained fermenters, hydrogenotrophic methanogens and syntrophs, while the vadose zone contained iron-reducing bacteria and methylotrophs. Conclusions: Because magnetic susceptibility is enhanced in and above the free phase oil plume, measuring it can be a promising tool for monitoring bioremediation.
Abstract Title:
Assessment of the Sensitivity of Various Bioparameters As Ecotoxicological Monitoring Tools During Sawdust Assisted Biorestoration of Diesel-Polluted Soil
Primary Author Block:
C. O. Onwosi1, J. N. Odimba1, V. C. Igbokwe1, C. J. Aneke2, T. N. Nwagu1, F. O. Nduka1, I. E. Eke1; 1Univ. of Nigeria, Nsukka, Nigeria, 2Enugu State Univ. of Technology, Agbani, Nigeria
Abstract Body:
Bioremediation have been reported and recognized as an effective method of restoring polluted soils to their original soil quality. As such, a number of monitoring tools have been identified as good indicators of the effectiveness of a bioremediation strategy. A number of biological parameters have been reported as effective monitoring tools, of which soil enzymatic activities has gained wider popularity as compared to other soil biological activities. Therefore, this study investigated a number of microbiological activities in the soil to serve as biomonitoring tools in assessing the ecotoxicity of diesel contaminated soil samples during the different periods of bioremediation. Sawdust, which served as organic amendment, was used for the biodegradation of artificial diesel-polluted soil samples. Soil microbial population, soil microbial enzymatic activities (catalase, lipase, dehydrogenase, urease, phosphatase and β-glucosidase), soil microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP), soil microbial respirometric index and total petroleum hydrocarbon (TPH) concentration were monitored to evaluate the efficiency of the bioremediation process. After a period of 56 days, total petroleum hydrocarbon content reduced from 14221 mg/kg to 270 mg/kg. However, to evaluate the efficiency of the sawdust assisted bioremediation, various statistical tools were employed including principal component analysis, which was used in selecting the most sensitive bio-indicators. It was observed that MBC, MBN, MBP and total hydrocarbon utilizing fungi (THUF) enumeration were the most responsive bioparameters. A positive relationship between TPH removal and the four most sensitive isolated bioparameters suggests that the use of other biological activities in the soil apart from the enzymatic activities in the soil, should not be greatly undermined as MBC, MBN, MBP and THUF have proven to be efficient monitoring tools for evaluating the efficiency of a bioremediation strategy.
Abstract Title:
Paenibacillus Sp., A New Plastic Degrading-Bacteria

Primary Author Block:
D. K. R. Bardají, V. S. Braz, A. F. F. Tonelli, J. A. S. Moretto, E. G. Stehling; Univ.e de São Paulo, Ribeirão Preto, SP, Brazil

Abstract Body:
Background: Plastics are man-made long chain polymeric molecules. The annual production of plastics has doubled over the past 15 years to 245 million tons due to their great physical and chemical properties, thus a large amount of plastic gets accumulated in the environment generating plastic waste ecological problems. The purpose of this study was to isolate bacteria from a waste disposal area with potential to degrade plastic. Methods: These bacteria were isolated in Ribeirão Preto, SP, Brazil using plastic discs as a carbon source and a minimal salt medium (MSM). After genomic DNA extraction, PCR reactions were performed to detect the alkB gene and bacteria that showed this gene were identified and incubated with plastic discs (polyethylene, polyurethane and polyvinyl chloride 5cm discs) and MSM (90mL) for 3 months. After incubation, lose weight measurement analysis, Fourier Transforms Infra-red (FT-IR) analysis, Scanning Electron Microscopy (SEM) analysis and antimicrobial susceptibility tests were performed to evaluate the biodegradation capacity and the resistance profile of the isolates. Results: Five bacteria were isolated, however, only one showed the alkB gene. This bacterium was identified as Paenibacillus sp. using the 16S rDNA gene sequencing. Plastic discs were used normally or chemically treated with tween 80, bleach and ethanol solution and incubated in MSM with the Paenibacillus sp. for 3 months. A significant difference in final weight compared to initial weight was assessed for the 3 types of plastic. Chemical changes were observed by FTIR. The appearance of new functional groups (carboxylic acids (3300-2500 cm⁻¹), esters (1210-1163 cm⁻¹) and ethers (1075-1020 cm⁻¹) and bond scissions were more evident for polyethylene films. SEM visualized physical changes, such as formation of pits and cracks, and bacterial colonization on the plastic surface in all cases. Paenibacillus sp. showed susceptibility to all antibiotics tested except for amikacin. Conclusions: Biodegradation experiments demonstrated the ability of Paenibacillus sp. to modify and colonize plastic. The resistance profile of the isolate showed a low antibiotic resistance providing and additional benefit for its use in bioremediation. Hence this bacterium can be used widely for biodegradation as a promising tool for the elimination of plastic from the environment.
Abstract Title:
Evaluation of An Alginate Immobilized Pahs Tolerant Mixed Consortium after A Time of Storage
Primary Author Block:
R. Pacheco, D. V. Cortés-Espinosa, A. E. Absalón; Inst. Politecnico Natl., Tlaxcala, Mexico
Abstract Body:
Background: The technologies for remediation of contaminated soil have shown that the use of bacteria and fungi in co-metabolism have been effective. Bioremediation is an attractive approach for cleaning up petroleum hydrocarbons because it is simple to maintain, applicable over large areas, eco-friendly, cost-effective and leads to the complete degradation of the contaminant. However, many factors affect the success of the bioaugmentation process, such as competition with native microorganisms and unfavorable adaptation to the environmental changes. Immobilization techniques have been probed to maintain the cell viability in many bioremediation processes (1, 2, 3, 4). In this work, we performed an evaluation a microbial consortium immobilized in alginate beads and storage for 22 months for degradation of PAHs in soil. Methods: Microbial consortium (Aspergillus flavus, Aspergillus nomius, Thrichoderma sperellum, Bacillus cerus, Pseudomonas aeruginosa, Klebsiella pneumonia, Kelebsiela sp., enothrophomonas maltophilia) was immobilized by encapsulation technique in alginate and was storage for 22 months ago at room temperature. The evaluation was in a solid state fermentation with contaminated soil (Phenanthrene (Phe):Pyrene (Py): 3000 ppm) during 60 d. Samples were analyzed at 5,10,15,20,25,30 and 60 d. Four different treatments were evaluated: (BA) Soil+immobilized consortium+PAHs, (BA-H) Soil+ immobilized consortium-PAHs, (BE) Soil+ PAHs, (BE) Soil- PAHs. The measurement parameters were heterotrophic activity, colony forming units and residual PAHs. Results: Plate count showed that the immobilized maintained 11% of the viability with 3.075x108 CFU/g of immobilized after 22 months. During the kinetics, the consortium remained viable 60 d and shows an increase of 2.8 x1010 CFU/ g of dry soil at 60 day, these results are comparative with the accumulated heterotrophic activity 20 mg de CO2 / g of dry soil during the 60 d of the kinetics while treatments without hydrocarbons only show 10 mg de CO2/ g of dry soil. The PAHs degradation capacity was probed whit 94% of total PAHs remotion at 60 d. The degradation of Phe and Py was evaluated separately. Phe was completely degraded while Py showed a degradation of 87% both at 60 d.
Conclusion: The alginate beads immobilized PAHs tolerant mixed consortium maintained the viability and degradation capacity after a period of 22 months of storage.
Abstract Title:
Influence of Methane Inhibitors and High Molecular Mass Electron Donors on Chlorinated Solvent Biodegradation

Primary Author Block:
M. Ivey, K. Finneran; Clemson Univ., Clemson, SC

Abstract Body:
Chlorinated solvent bioremediation encompasses a number of combined microbial and chemical reactions that oxidize or reduce the contaminant(s) of concern. In the case of trichloroethylene (TCE), many approaches rely on adding electron donors to stimulate chlororespiration, in which cells gain energy to grow by sequentially reducing TCE to ethene. In recent years, the idea that TCE could be reduced by inhibiting methane production to stimulate dechlorination has been put into practice by vendors. The theory is that if methane production is inhibited, electrons will be redirected to chlorinated solvent reduction for complete dechlorination. However, if methanogenesis is inhibited, then microbial activity that is key in reducing chlorinated solvents may, or may not, occur. Additionally, adding carbon substrates to the subsurface rarely targets a single microbial population, and several microbial groups respond to electron donor amendment. The purpose of this research is to evaluate the influence of methane inhibitors on chlorinated solvents by using electron donors in various concentrations. Electron donors include plant-based essential oils, lactate, and statins. The work will demonstrate that inhibiting methanogenesis alone may not expedite dechlorination, and the broader impacts on the microbial community that are central to reducing TCE are larger than this single reaction. It is anticipated that using electron donors at near stoichiometric concentrations will help control methanogenesis while facilitating complete dechlorination. Data suggest that experiments containing high molecular mass electron donors may be more effective when paired with a methane inhibitor than when applied alone, specifically for lactate and emulsified vegetable oils. However, the addition of some amendments intended to be methane inhibitors may be ineffective at controlling methane production, and in some cases methanogenesis increased due to the other materials present with statins (e.g. yeast). One statin used appears to not be inhibiting methane production at all, but two plant-based essential oils could be effective at controlling methanogenesis during dechlorination. Although the vendors market these with added electron donors, each of the materials acts as an electron donor on its own. The statin may be less effective because of a misreported statin content in the red yeast rice carrier. Future analysis will determine whether the product contains a high enough percentage of statin compounds to be effective at controlling methanogenesis.
Abstract Title:
Characterization and Manipulation of S. Alterniflora Microbiomes for Increased Oil Remediation Efficiency

Primary Author Block:
C. M. Gardner, C. K. Gunsch; Duke Univ., Durham, NC

Abstract Body:
Millions of gallons of oil spilled into the Gulf of Mexico during the Deepwater Horizon Oil Spill of 2010, potentially disrupting multiple ecosystem levels across the Gulf Coast of the United States. One of the most promising strategies for large scale oil removal is in situ microbial bioremediation within coastal plants and sediments, which employs endogenous microbes naturally capable of metabolizing the compounds found in oil. Although some microbes within oil contaminated environments have been isolated from plants and characterized for their capacity to degrade PAHs, little is known about in situ microbiome structures and metabolic pathways involved in the degradation of PAHs by these communities. In salt marsh sediments, such as those along much of the Gulf Coast, anaerobic and aerobic biodegradation may co-occur in the rhizosphere or within plant tissues and involve an array of complex syntrophic degradation processes. Thus, salt marsh sediments may be selecting for prokaryotes and fungi carrying genes involved in hydrocarbon degradation and represent diversity hot spots for PAH-degrading genera. Accurate characterization of these plant salt marsh microbiomes is essential for characterizing existing and novel microbial PAH degraders as well as understanding the diverse metabolic processes involved in oil bioremediation. However, co-amplification of chloroplast and mitochondrial DNA is a common and significant hurdle when characterizing in plant-based metagenomic libraries. To address this issue, endophytic and rhizophytic prokaryotic and fungal communities associated with oil and non-oiled S. alterniflora roots and leaves were characterized using Illumina MiSeq sequencing. Prokaryotic universal PCR primers were used to target the multiple regions of the 16S rDNA of bacteria and archaea (i.e., V1-V2, V3, V3-V4, and V5-V6), and universal PCR primers targeting the fungal ITS1 region. The construction of metagenomic libraries was optimized by incorporating PNA and C3 probes that prevented the co-amplification of chloroplast and mitochondrial DNA from plant tissues. Co-amplification of chloroplast and mitochondrial DNA decreased by more than 95% with the addition of custom PNA and C3 blocking primers, resulting in the detection of an additional 1500 bacterial OTUs. Additional work is being conducted to identify specific PAH-degrading genes expressed in the presence of oil as well as tentatively link HP-degrading genes expression level to the taxonomic data.
Abstract Title:
Macondimonas Diazotrophicus: A Representative of A Novel Gammaproteobacterial Family of Globally-Distributed, Sediment-Associated, Keystone Oil Degraders

Primary Author Block:
S. Karthikeyan1, L. M. Rodriguez-R1, P. Heritier-Robbins1, W. A. Overholt1, J. K. Hatt1, J. C. Spain2, M. Huettel3, R. Rosselló-Móra4, J. E. Kostka1, K. T. Konstantinidis1;  1Georgia Inst. of Technology, Atlanta, GA, 2Univ. of West Florida, Pensacola, FL, 3Florida State Univ., Tallahassee, FL, 4Inst. Mediterrani d’Estudis Avançats, Esporles, Spain

Abstract Body:
Modeling crude oil biodegradation remains a challenge due, in part, to the lack of appropriate model organisms. Our previous analysis of time series metagenomic data from beach sands affected by Macondo oil in Pensacola Municipal Beach, FL (Rodriguez-R et al., ISME 2015) revealed a high abundance of nitrogen fixing genes (namely nifH). Using targeted population reconstruction techniques, we have succeeded in reconstructing an almost complete genome that included the abundant nifH gene, together with other genes for hydrocarbon degradation, methanotrophy, urea metabolism, and biosurfactant production required to thrive under the often nitrogen-limited, oil-perturbed ecosystems. The relative abundance of the genome bin rapidly increased from no detectable levels in the clean/pre-spill samples to 29% of the entire community in oiled samples, returning to undetectable levels in the recovered sediments. 16S rRNA gene sequences of this genome were also and almost exclusively, found in oiled/hydrocarbon contaminated sediments across the globe, including the Prestige oil spill in the Galicia coast and in Cape Hallett in Antarctica (following an oil spill incident), and were undetected in clean or water-column samples. Metagenome-guided isolation efforts yielded a rod shaped bacterium that showed 99.8% genome-aggregate average nucleotide identity (ANI) to the previously identified population bin. Whole genome comparisons to available genomes revealed that our isolate represents a novel, deep-branching species within the Gammaproteobacteria, for which we propose the name Macondimonas diazotrophicus. To further understand how M. diazotrophicus responds to oil and its growth requirements, we have employed advective-flow chambers, which mimic in-situ pressure gradients in saturated beach sediments, inoculated with weathered Macondo oil. Our preliminary results showed that Macondimonas was strongly enriched during these laboratory mesocosms under different levels of oxygen and water saturation conditions. The ecological distribution and metabolic versatility of M. diazotrophicus, coupled to its abundance patterns during oil biodegradation, make it a promising organism for oil bioremediation efforts.
Do Microbes Have Memory? Repeated Exposure to Emulsified Vegetable Oil May Increase Degradation Ability of Native Microbial Communities

Primary Author Block:
K. McBride1, S. Jagadamma1, N. Daliang2, J-W. Moon3, C. Paradis1, D. Joyner1, T. Mehlhorn3, T. C. Hazen1; 1Univ. of Tennessee Knoxville, Knoxville, TN, 2Univ. of Oklahoma, Norman, OK, 3Oak Ridge Natl. Lab., Oak Ridge, TN

Abstract Body:
Background: Microbial “memory response” is the idea that microbial communities will degrade a substrate more rapidly if it has been exposed to it multiple times. This novel idea has the potential to increase the efficiency of many commonly-used bioremediation techniques. In order to test this concept, anaerobic microcosm experiment was conducted for 150 days using sediment and groundwater from a low-contamination aquifer at the Oak Ridge Field Research Center which had been previously amended with an emulsified vegetable oil (EVO) injection years before. Methods: Four groundwater wells from the same site were used to create the microcosms—two of the wells were directly downstream from the previous injection of EVO, and the other two were upstream and unexposed to EVO. All microcosms were amended with EVO, and changes in both microbial communities and geochemical properties were compared to see if the rate of degradation was faster in those that had already been exposed to EVO. Gas chromatography was used to measure CO2 and CH4 production in the microcosms at several time points, while ion chromatography measured levels of acetate, nitrate, and sulfate in the water. ICP-MS was also utilized to measure trace metals found in the water and sediment. To analyze microbial communities, DNA was extracted from both microcosm sediment and groundwater followed by 16S rRNA sequencing. Results: Results showed that after EVO addition, CH4 and CO2 were produced in both upstream and downstream samples at the same rate; similarly, nitrate and sulfate were also consumed at the same rate. However, acetate formed by EVO degradation was produced more rapidly and in much higher abundance in downstream wells. 16S data indicated that the relative abundance of known sulfate-reducing taxa, including those from the family Desulfobacteraceae, Desulfovibrionaceae, Geobacteraceae, and Desulfobulbaceae, increased and peaked around 30 days after EVO amendment, however, abundance was higher in downstream samples. Detrended correspondent analysis of OTU tables show that throughout all time points, there is a significant difference in the taxonomic community and population relative abundances between upstream and downstream wells at each timepoint. Conclusions: This data indicates that perhaps degradation occurs at the same rate in both previously exposed and un-exposed samples, however the abundance of relevant degrading-species—and therefore overall reduction ability—may be higher in the previously exposed samples.
Abstract Title:
Immobilization of A Consortium for Bioremediation of Contaminated-Hydrocarbons Soil

Primary Author Block:
D. V. Cortés-Espinosa, A. Moreno; Inst. Politecnico Natl., Tlaxcala, Mexico

Abstract Body:
Background: A large number and variety of microorganisms that habit contaminated soil, have the ability to tolerate and even mineralize a wide variety of polycyclic aromatic hydrocarbons (PAHs). These microorganisms are important for their application in bioremediation systems of contaminated soils by bioaugmentation, however many times these microorganisms are displaced by the same native microbiota; so, it is necessary to look for some strategy to ensure the survival of these microorganisms in the soil during the biodegradation process. The techniques of immobilization are used to protection and adequacy of the inoculum in a process of bioremediation. In this study, the immobilization of a microbial consortium was performed by adsorption using corn stover as support and substrate and this was evaluated for their capacity to remove PAHs and diesel in a contaminated soil by solid culture.

Methods: The consortium was formed by Trichoderma asperellum, Aspergillus nomius, Aspergillus flavus, Klebsiella pneumoniae, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella sp and Stenothrophomonas maltophilia and the matrices for immobilization were sterile corn stover and barley straw. Proportion of inoculum and agroindustrial waste were 1:1 (w/v), agroindustrial waste was mixed until absorbed the inoculum in a mixing drum. The drying process was done in trays at room temperature for 48 h. Evaluation of consortium immobilized was performed in microcosm solid culture systems using contaminated soil (3000 ppm of phenanthrene and pyrene (1:1)). Incubation time was 30 d at 37°C. Response variable were PAHs removal and CO2 quantification as an indirect measure of growth. Results: The results obtained suggest that the immobilized maize, reaches a higher percentage of PAH removal (53.69%), this could be due to the fact that the microorganisms had a greater reach to the contaminants within the crop matrix, also a greater metabolic activity of the immobilized in maize than in barley, suggesting that the consortium has a larger carbon source that allows microorganisms to be active in the soil. The consortium immobilized in barley shows 45% of PHAs removal. Conclusion: The microbial consortium immobilized in maize stover is an excellent possibility to improve a process of bioremediation of PAHs-contaminated soil, since it is a protection to the inoculum but in turn, allows the consortium release from the initial day. Another advantage using of agroindustrial waste as immobilization matrix is that it confers porosity to soil, allowing the oxygen diffusion.
Abstract Title:
Genomic Insights and Functional Validation of Bacterial Isolates for Rhizosphere Mediated Bioremediation of Polyaromatic Hydrocarbons

Primary Author Block:
P. Pandey; Assam Univ., Silchar, India

Abstract Body:
The genomic characteristics of bacterial strains capable to degrade higher molecular weight polyaromatic hydrocarbons (PAHs) were investigated. Five bacterial strains, isolated from crude oil contaminated soil of Assam (India) including, Serratia marcescens S2I7, Bacillus subtilis SR1, Klebsiella pneumoniae AWD5, Pseudomonas aeroginosa DBC and Alcaligenes faecalis BDB4 were selected, because of their excellent PAH degradation, and plant growth stimulation abilities. The isolate S2I7 and SR1 could degrade benzo(a)pyrene up to 80.4% and 72.9% respectively after 21 days. The strain AWD5 degraded 49.1% pyrene, 36.6% chrysene and 50.5% benzo(a)pyrene after 9 days as estimated by GC-MS analysis. Whereas DBC and BDB4 showed degradation of pyrene up to 52.9% and 48.5% respectively after 6 days. The strains also showed resistance to heavy metal (cadmium) and produced bio-surfactant for solubilization of hydrophobic PAHs. The genomes of the strains were sequenced using Illumina paired-end library with an average insert size of ~400 bp. The genomic sequence of the strains revealed versatility for degradation, emulsification and metabolizing of aromatic compounds. Analysis of cluster of orthologous group (COG) revealed that S2I7, DBC, BDB4 has significantly higher gene abundance for aromatic compound degradation, stress response and cell motility. Bacillus subtilis SR1 had genome of 4093698 bp with 44.01% GC content while Serratia marcescens S2I7 had largest genome of 5241555 bp with 60.1% GC content. The genomes had diverse group of dioxygenase genes of aromatic ring hydroxylating dioxygenase (ARHD) family, however genome of S2I7 reveals the presence of several mono-oxygenase genes also that are predicted to be involved in degradation pathways. KEGG analysis of the selected genomes predicted and validated the involvement of both dioxygenase and mono-oxygenase enzyme system simultaneously for degradation of complex compounds. The genomic analysis of these bacterial strains provide a better understanding of the complexity of bacterial catabolic pathways for degradation of polyaromatic hydrocarbons, and their use for rhizosphere-mediated bioremediation. Key words: Genome, PAHs, Heavy metals, KEGG, Dioxygenases.
Session Number: AES06 - Bioremediation, Biodegradation, Biofouling, and Biocorrosion II
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Session Primary Track: Applied and Environmental Science
Abstract Control Number: 6771
Poster Board Number: SUNDAY - 842

Abstract Title:
Degradation of Alkane Compounds in Kerosene by Marine Bacteria and Bacterial Consortia with Additional Nutrients

Primary Author Block:
S. Kim1, J. Kim2, Y-H. Chung2, S. Kim2, H-Y. Kahng1; 1Sunchon Natl. Univ., Suncheon, Korea, Republic of, 2Korea Basic Sci. Inst., Daejeon, Korea, Republic of

Abstract Body:
Background: This study aimed to construct the most effective marine bacterial consortium using the sets of three bacterial species Brevibacterium frigoritolerans SHD-34, Pseudomonas brassicacearum SHG-4, and Pseudomonas taeanensis MS-3 (2010, 60: 2719-2723, International Journal of Systematic and Evolutionary Microbiology), which are applicable to the bioremediation of marine environment.

Methods: Several sets of bacterial consortia were constructed using the three bacteria. Their oil degradation capabilities were measured by GC-MS. Additional six carbon (glucose, lactose, maltose, fructose, sucrose and citrate) and/or four nitorgen (NH4Cl, peptone, urea, and yeast) sources were investigated for the effect on their degradation abilities. All the biodegradation tests for petroleum oils were performed at 1% concentration. Results: Four sets of bacterial consortia (KII-1, KII-2, KII-3, and KIII) were constructed and tested for their petroleum oil degradation abilities, resulting in the selection of two bacterial consortia, KII-1 (a combination of SHD-34 and SHG-4) and KII-2 (SHD-34 and MS-3) based on the biodegradation abilities. KII-1 and KII-2 were found to perform more than 90% kerosene degradation. In GC-MS analysis, alkane compounds (C7 ~ C19) in kerosene were almost completely degraded in the range of 99.36 to 100% degradation rate with glucose or lactose by B. frigoritolerans SHD-34, which is very similar to that of consortium KII-2. P. taeanensis MS-3 exhibited very different degradation rate. Heptane(C7) ~ decane(C10) were degraded to the range of 90.1 to 100% with glucose by P. taeanensis MS-3, while undecane(C11) ~ nonadecane(C19), to the range of 51.5 to 71.0% with the same carbon source. Alkane compounds (C7 ~ C19) were degraded to the range of 95.3 - 100% with lactose by MS-3. Consortium KII-1 exhibited very similar n-alkane compounds degradation rate (=more 100%) in the absence or presence of lactose, maltose and D-fructose, while glucose and citrate rather reduced the degradation rate. In effect of nitrogen sources, C7 ~ C19 were almost completely degraded to 100% degradation rate with yeast by B. frigoritolerans SHD-34, P. taeanensis MS-3, KII-1, and KII-2, suggesting the most effective one of the four nitrogen sources to enhance the kerosene degradation rate. Conclusions: Two marine bacterial consortia, KII-1 and KII-2 were found to have an outstanding kerosene degrading ability, suggesting they are considered applicable to the effective bioremediation of marine environment contaminated with petroleum oils.
Trichlorfon (TCF) is one of the most widely used organophosphate pesticides in agriculture. However, limited information is known about the biodegradation behaviors and kinetics of this pesticide at present. Recently we build up a novel TCF biodegradation pathway in Bacillus tequilensis and demonstrate that hydrolyzation and P-C bond cleavage are the two main reactions during the TCF biodegradation by GC-MS analysis. We further showed four important intermediates that are newly investigated under the conditions that TCF is supplemented as sole phosphorus and sole carbon-phosphorus sources. These novel findings indicate that oxidation and reductive dechlorination are also the two main reactions besides hydrolyzation during TCF biodegradation. As a model system, Bacillus tequilensis were cultured in TCF contaminated soils and the DGGE were employed in characterizing the dynamics of soil microbial communities in relation to changing environmental factors. In sterilized and unsterilized soils, TCF degradations were both significantly affected (p < 0.05) by culturing Bacillus tequilensis, reaching the highest of 100% in 6 days. The DGGE results revealed that the bacterial community Proteobacteria sp., Firmicutes sp. and Actinobacteria sp. were affected by the inoculation of TCF and exogenous bio-degrader Bacillus tequilensis. However, the inoculation of Bacillus tequilensis mitigated the effects of TCF to the fungal community Ascomycota sp. and Basidiomycota sp. in soils. Our identification of the specific biodegradation reactions and inoculation of Bacillus tequilensis in soil may give us approaches to understand the TCF degradation behaviors in microorganisms. The model strain Bacillus tequilensis used in this study mitigate the negative effects to soil microbial community structures, and exhibit significant bioremediation potential in the TCF contaminated environment.
Abstract Title:
The Gut Microbiota of Housefly Larvae (Musca Domestica) Houses Monensin Degrading Bacteria: A Potential Source for Biotechnological Exploitation

Primary Author Block:
H. Li, Q. Wan, C. Wang, S. Zhang, S. Su, B. Pan; China Agricultural Univ., Beijing, China

Abstract Body:
Monensin (MON) are ionophore antibiotics (IPAs) widely used in broiler feed to promote growth and control coccidiosis. Most of the ingested IPAs are excreted in chicken manure. Because poultry litter is commonly used to fertilize agricultural fields, ionophore residues in litter have become contaminants of emerging concern. Vermicomposting of livestock manure using housefly larvae is a promising biotechnology for waste reduction and control of antibiotic pollution. This study aimed to develop a HPLC with post-column derivatisation method to quantify monensin in larvae substrate with chicken manure and wheat bran, and explored the efficiency on monensin attenuation during a 12-day laboratory scale vermicomposting via housefly larvae (Musca domestica). A 95% reduction in MON concentration was achieved within 4 days in the treatment group, while it took 12 days to remove more than 94% of MON in control group. The temperature and pH were positively linked to the level of residual MON. We also isolated and accessed the MON-degrading capacity of gut bacteria from the gut of housefly larvae using MON-selective media, which containing only the MON as carbon source for bacterial growth. Two isolates were obtained, which were identified by Illumina MiSeq 16S rRNA sequencing as Alcaligenes faecalis and Stenotrophomonas maltophilia. In conclusion, the gut microbiota of housefly larvae houses monensin degrading bacteria with bioremediation potential.
Currently In Korea, dredged soil generated by port development and operation is mostly landfilled in coastal landfills or partly in the ocean. The dredging site is close saturation. Due to environmental problems, the construction of the dredged dump site is difficult. For the treatment of dredged soil, it is urgent to develop application for purification treatment and recycling of pollutants. The bioremediation system for polluted marine sediment was developed to resolve these problems and propose a solution to recycle of the sediment. In this study, a pilot plant was constructed and installed to remediate contaminated dredged soil. The dredged soil has been artificially contaminated by organic contaminants such as Total Petroleum Hydrocarbons (TPH), Poly Aromatic Hydrocarbon (PAH) and Trichloroethylene (TCE). The pilot-plant has been operated for 6 months (June 2017 to December 2017) and consisted of 4 aerobic tanks (each tank; 7 tons). The bioremediation system can be used exclusively or continuously according to the type of pollution source and concentration of pollution. Organic substance pollutants are biologically treated by bacteria mixture. The bacteria mixture was included was Pseudomonas sp., Rhodococcus sp., and was applied to the pilot-plant. In optimal operation conditions, TPH removal efficiency had shown more than 90 % (Initial; 119,795 mg/kg) for 16 hours. In case of PAH, removal efficiency was 96.8 % for 16 hours at initial concentration 136.0 mg/kg and TCE was not detected. The results of the pilot scale study confirmed the feasibility of the site for the contaminated dredged soil remediation.
Abstract Title:
Isolation and Characterization of Mesophilic and Moderate Thermophilic Dibenzoferan Degrading Bacteria from Vietnamese Soil

Primary Author Block:
T. Ngoc Thi1, L. Ha Thanh1, M. Shintani1, N. Hoang Loc2, K. Kimbara1; 1Shizuoka Univ., Hamamatsu, Japan, 2Coll. of Sci., Hue Univ., Hue, Viet Nam

Abstract Body:
Dioxins and dioxin-like compounds are compounds known to be toxic, carcinogenic, and persistent environmental pollutants. Microorganisms, especially bacteria, play an important role in the biodegradation of dioxins in natural environments. Dibenzoferan (DF) has been used as a model compound for the investigation of the bacterial degradation of dioxins. In this study, we isolated and characterized DF-degrading bacteria in soil of A Luoi District (Viet Nam), since this area is polluted by dioxin occurrence. We used enrichment culture using a mineral salts medium (W medium) supplemented with DF as the sole source of carbon and energy for the isolation of DF-degrading bacteria from five different soil samples at 30°C and 50°C. We isolated eight DF-degrading bacteria at 30°C and four degraders at 50°C. The isolates were identified by sequences of their 16S rRNA gene, and all these strains were identified as genus Paenibacillus. For further characterization, we chose representative strains, 1-10 and 4B1, from the isolated degraders at 30°C and 50°C, respectively. The 16S rRNA gene sequences of 1-10 and 4B1 are 99% identity with a DF-degrading bacterium, Paenibacillus sp. YK5 isolated from Japanese soil (Iida et al., 2006, Arch Microbiol 184: 305-315). The relative growth rate on W medium with 1000 and 1500 mg/L DF were very similar but the final cell density with 1500 mg/L DF was higher than that with 1000 mg/L DF. The growth rate of isolates were higher than strain YK5 on W medium added 1000 mg/L DF. Strain 4B1 could grow on W medium with 1000 mg/L DF at temperature from 30-50°C but not at 55°C. The optimum growth temperature of 4B1 was 40-45°C after 30 h. The metabolic capability of isolated strains was investigated for several aromatic hydrocarbons. The isolates could grow on naphthalene, pyrene, benzoic acid, salicylate; only 1-10 strain could grow on gentisate and no strains could grow on catechol, either of which were known to be putative intermediate compounds of the salicylate. These results suggested that the DF metabolic pathway of 1-10 strain could be via salicylate and gentisate. The genes involved in DF degradation dbfA, dbfB, dbfC of isolates have high identity (99%) with Paenibacillus sp. YK5. This study showed that 1-10 and 4B1 strain had similar 16S rRNA, however their degradation pathways of DF could be different. The isolates would be potentially used in bioremediation of aromatic compounds and dioxins.
Abstract Title:
Real-Time PCR Assay for Evaluating the Distribution of Mercury-Resistant Bacilli in Environmental Soils

Primary Author Block:
K. Matsui1, G. Endo2; 1Kindai Univ., Higashiosaka, Japan, 2Touhoku Gakuin Univ., Tagajo, Japan

Abstract Body:
Bacillus megaterium strain MB1, an mercury-resistant (HgR) isolate from mercury-polluted sediment in Minamata, Japan, is resistant to organomercurials and inorganic mercury salts, and carries broad-spectrum mercury resistance (mer) genes on a Tn3-family of replicative transposons (TnMERI1). In a previous culture-dependent analysis, we showed that mer genes were distributed worldwide in Bacilli with the dissemination of TnMERI1-like transposons1). To understand the mobile nature of mer genes and the ways in which HgR Bacilli colonize new environments, it is necessary to develop a reliable quantitative method for identifying mer genes in environmental soil samples. Here we developed a real-time PCR assay to quantify the mercury reductase (merA) and organomercury lyase (merB1) genes in TnMERI-like transposons. First, to ensure the specificity of PCR detection, PCR primers and Taqman probes were designed and tested. The best primer-probe pair allowed us to quantify 25 copies of the target genes in a PCR reaction mixture. Second, we evaluated the efficiency of DNA recovery in different types of soils (e.g., sand, silt, humic). The cultivated B. megaterium MB1 cells were spiked into each type of soil as an internal standard. The efficiency with which spiked cells were recovered varied from 24% to 60%, depending on the type of soil. These results indicate that 1×104 copies of a mer (A or B1) gene per 1 g soil is the detection limit for environmental soil sample analysis by this method. Finally, we evaluated the amount of merA and merB1 in environmental soil samples collected from different regions. We were able to quantify mer genes in only 7 out of 70 samples. The other 63 sites, including the site containing culturable HgR Bacilli, provided quantities of genetic material that were below the detection limit of the real-time PCR assay. However, further cultivation of the soils under nutrient-rich conditions allowed the detection of mer genes via PCR. These results suggest that dormant HgR Bacilli were distributed in various environments at a density of less than 1×104 cells per 1 g soil, and were activated under suitable environmental conditions. This finding prompts us to propose in situ molecular breeding of widely distributed HgR Bacilli for bioremediation without the use of genetically modified microorganisms.
Abstract Title:
Field Metagenomics of Microbial Community Involved in Bioremediation of Crude Oil-Polluted Soil
Primary Author Block:
C. B. Chikere, C. C. Ezekoye, G. C. Okpokwasili; Univ. of Port Harcourt, Port Harcourt, Nigeria
Abstract Body:
Background: The microbial community structure and dynamics in a crude oil-polluted soil undergoing landfarming was monitored over a 56-day period to evaluate their role in different stages of bioremediation in order to develop better remediation procedures. Hydrocarbon polluted soil was collected from the site in Ibaa Community, Rivers State Nigeria, on days 1, 9, 29, 36 and 56 during remediation. Methods: The microbial community structure at different stages of remediation was compared to an unpolluted soil collected 70 meters away from the site undergoing remediation. Metagenomic DNA samples from the soil were sequenced using Illumina MiSeq and analyzed using the QIIME pipeline. Soil total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) were quantified as bioremediation progressed with GC-FID. Results: The baseline TPH pre-remediation was 9,146.6 mg/kg which reduced to 677.2 mg/kg on day 56 while the PAHs concentration reduced from 3,248.8 mg/kg pre-remediation to 356.5 mg/kg on day 56. Alpha diversity analysis using Shannon and inverse Simpson diversity indices revealed that the sample collected on day 36 of bioremediation had the highest diversity and richness of organisms while that taken on day 56 had the least diversity. Principal coordinate analysis showed that the polluted soil-pre remediation, the samples taken on days 1 and 9 during treatment clustered together. A gradual shift from the abundance of Actinobacteria to the abundance of Proteobacteria was noticed during the preliminary stage of remediation (days 1 - 9) which became very rapid from the mid to late stages of the remediation process. Second order binomial model revealed that TPH loss on days 9, 29, 36 and 56 were 29.07%, 51.69%, 71.80% and 92.16%, respectively. Conclusions: This study demonstrated that bacteria of the phylum Proteobacteria played key role in the remediation process immediately the polluted soil was amended as their increasing abundance correlated with hydrocarbon removal from the site.
Biodegradation of Chloroethene Compounds by An Indigenous Mixed Microbial Consortium

Abstract Body:
Background: Tetrachloroethene (PCE) has been considered as recalcitrant to microbial biodegradation due to its persistence in aerobic conditions. However, in anaerobic environments, PCE can be used as the sole carbon source by microorganisms. Here we evaluate biodegradation potential of an indigenous microbial consortium.

Methods: We assessed tolerance of the indigenous mixed microbial consortium to PCE (0.01 mM to 1.8 mM). Batch experiments were conducted to assess biodegradation ability of PCE alone (at 0.6 mM) and in combination with lactate (PCE 0.6 mM, lactate 8.5 mM). Chloroethenes (CEs) biodegradation was monitored using gas chromatography (GC) analysis. Additionally, next generation sequencing (NGS) analysis was performed on environmental samples and enrichment cultures, and NGS data analysis was done using bioinformatic tools.

Results: Initial results indicated that during anaerobiosis, the consortium was highly tolerant to PCE with minimum inhibitory concentration (MIC) of PCE at 1.2 mM. GC analysis results showed biotransformation from PCE to trichloroethene (TCE), cis-1,2-dichloroethene (cDCE), trans-1,2-dichloroethene (tDCE), 1,1-dichloroethene (1,1-DCE), vinyl chloride (VC) and ethene as final products. This was probably due to a reduction process, as redox potential (Eh) changed from 308 mV to 22 mV. Over a period of 90 days, 38% of average PCE degradation was achieved when PCE was the sole carbon source. While 10% of average PCE degradation was observed in the presence of lactate. Higher degradation percentages were observed for the daughter products (i.e., 52% of TCE, 60% cDCE, 100% tDCE on average) in the absence of lactate. In contrast, when lactate was added, higher average degradation was observed for 1,1-DCE (56%). The NGS analysis revealed that Proteobacteria and Firmicutes dominated the batch experiments containing both PCE and lactate; whereas Proteobacteria, followed by Bacteroidetes and Actinobacteria dominated the batch experiment with PCE alone. At the genus level, Hydrogenophaga sp., Lysobacter sp., Flavobacterium sp., Rhodanobacter sp., Clostridium sp., Anaerolinea sp., Burkholderia sp., Listeria sp., Dehalobacter sp., Clostridium sp., Flavobacterium sp, were found to dominate these communities.

Conclusions: Degradation of PCE occurred when PCE serves as the sole carbon source. Members of identified genera have been shown to have the ability to degrade CEs and can, therefore, be used as a starting point for bioremediation processes.
Use of Box-Behnken Design in the Optimization of Total Petroleum Hydrocarbon Removal During Composting of Diesel-Polluted Soil

C. J. Aneke; Enugu State Univ. of Technology, Agbani, Nigeria

Nigeria is one of the major oil-producing countries and as a result, is faced with various forms of pollution emanating from crude oil exploration, transport and utilization. This has hampered the agricultural activities and endangered most aquatic organisms owing to the toxicity associated with the pollutants.

To this end, the current study was undertaken to optimize the composting materials during the bioremediation of the polluted soil. The optimization of the organic amendments was performed using the Box-Behnken statistical experimental design. This design consisted of 15 runs of 3 factors (poultry droppings, sawdust and grass trimmings) at 3 levels which included three central points to analyze the model robustness. The rate of total petroleum hydrocarbon removal, lipase and dehydrogenase activities were considered as the experimental responses for monitoring of the effectiveness of the biodegradation. The outcome of the experiment indicates that a mixture of grass trimmings (158.2 g), poultry droppings (50g) and sawdust (602.7g) resulted in best total hydrocarbon removal rates. The dehydrogenase and lipase activities of the soil microbes were effective biomonitoring tools during the remediation. This study clearly shows that cheap organic amendments are indispensable in the removal of pollutants from the environment.
Abstract Title:
Freshwater Microbial Community Responses to Different Oil Types
Primary Author Block:
T. Butler1, P. Webb1, M. Brenemann1, J. SantoDoming2, S. Techtmann1; 1Michigan Technological Univ., Houghton, MI, 2Michigan Technological Univ., Cincinnati, OH
Abstract Body:
There is great concern regarding the potential impact of an accidental release of oil from pipelines transporting oil across freshwater systems (Dakota Access Pipeline and the Enbridge Line 5 pipeline, which crosses the Great Lakes at the Straights of Mackinaw). The extreme sensitivity of freshwater systems including the Great Lakes would make an oil spill devastating to the environment. Due to the lack of knowledge of freshwater microbial community responses to oil spills, cleanup efforts of an oil spill may be hindered. We have undertaken a microcosm-based study to examine the impact of crude oil on microbial community structure. The primary hypothesis of this research is that the type of oil is a major influence on the environmental impacts of oil spills. Additionally, we are interested in identifying the bioremediation potential of microbial communities in the Great Lakes. This hypothesis will be tested through analyzing the impact two oil types (Diluted bitumen - a heavy oil derived from Canadian Oil Sands; and Bakken crude oil - a light oil extracted from the Bakken Shale in North Dakota). Microcosms were prepared from seven locations throughout the Great lakes including three in the straights of Mackinaw near the Enbridge Line 5 pipeline. Samples were taken from microcosms to characterize shifts in microbial community composition using high-throughput 16S rRNA sequencing. Samples were also collected to characterize the rates of hydrocarbon degradation in these microcosms. We expected that the Bakken oil would result in a more rapid community change due to it being a lighter API gravity oil that is presumably more easily broken down by bacteria as compared to the diluted bitumen oil which is a heavy API gravity oil. It was also expected that a distinct microbial community is required to degrade each of the two oils. Our findings showed community diversity decreased in response to oil addition. The addition of oil resulted in an increase in the relative abundance of known oil-degrading taxa. Moreover, the data showed that the different oil types selected for a distinct microbial community composition. These results indicate that there is a robust response to these oil types by freshwater microbial communities. This work will greatly contribute to better understanding of freshwater oil biodegradation and impacts of an oil spill in these sensitive freshwater environments. Understanding of microbial responses to these oil types across the Great Lakes and into other freshwater settings will also contribute to informed spill response strategies.
Abstract Title:
Comparative Proteomic Analysis of Burkholderia Strains Isolated from Savannah River Site, Under Uranium Stress
Primary Author Block:
M. Agarwal1, R. Jaswal1, R. Rathore1, O. Sharma2, R. Singh3, J. Seaman4, A. Chauhan1; 1Florida A&M Univ., Tallahassee, FL, 2Florida State Univ., Tallahassee, FL, 3Florida State Univ., Tallahassee, FL, 4Univ. of Georgia, Aiken, GA
Abstract Body:
USA has an enormous radioactive contaminated surface; the area is bigger than the great lakes combined area. One of the most frequently found contaminants is Uranium (U). Uranium is used as a fuel source for nuclear power plant and nuclear weapons and thus produced Uranium waste and its byproducts are discharged into the water streams. U is a serious threat to environment and can also become a part of food chain, bioaccumulate and thus become a human health issue. Human body organs like kidney, brain, liver and heart are affected by exposure to U and its long term exposure has been a big cause of lung cancer. However, the soil microbes have adapted to resist and grow under U toxicity. Some of these microbes can also precipitate the mobile Uranium (U(VI)), so that it no longer remains an active threat to the environment. Uranium may undergo various other, poorly understood, transformations owing to the microbial community in the soil. U-contaminated soils were collected from the Savannah River Site (SRS) to study the microbial diversity, resistance mechanisms and biotransformation of U. Metagenomic analysis of the soils is being performed to study the microbial diversity. Microbes were isolated from these soils by traditional enrichment cultures (containing U). Of the isolated strains, two bacteria (Burkholderia strain SRS-S-25-2016 and Burkholderia strain SRS-S-46-2016) demonstrated maximum U resistance and were further studied for protein expression by proteomics under stress free and U-stress conditions. Proteomics study revealed that up-regulation of proteins which are involved in response to metal binding protein, DNA damage response, proteins with transferase activity of methyl and phosphate group, and stress condition.
Dimethyl Sulfoxide (DMSO) Reductase is Required for Iodate Reduction by Shewanella Oneidensis

Abstract Title:

Dimethyl Sulfoxide (DMSO) Reductase is Required for Iodate Reduction by Shewanella Oneidensis

Primary Author Block:

H-D. Shin1, Y. Toporek1, J. Mok1, B. Lee2, M. Lee3, T. DiChristina1; 1Georgia Inst. of Technology, Atlanta, GA, 2Pacific Northwest Natl. Lab., Richland, WA, 3Savannah River Natl. Lab., Aiken, SC

Abstract Body:

Background: Microbial iodate (IO₃⁻) reduction is a major component of the iodine biogeochemical reaction network in iodine-contaminated subsurface environments. The liquid extraction and trapping of volatile iodine gases via microbial iodine biotransformation is an attractive method for remediation of radioactive iodine-contaminated subsurface environments. We previously discovered that the metal (but not nitrate) reduction pathway is required for IO₃⁻ reduction by the metal-reducing bacterium Shewanella oneidensis. The terminal IO₃⁻ reductase, however, has yet to be identified. In the present study, a variety of metal (mtr) reduction pathway paralog mutants of S. oneidensis were examined for IO₃⁻ reduction activity to identify candidate enzymes catalyzing the terminal IO₃⁻ reduction reaction.

Methods: Six S. oneidensis mtr paralog mutants (ΔmtrA-ΔmtrDEF, ΔmtrA-ΔdmsEF, ΔmtrA-ΔSO_4360, ΔmtrF, ΔdmsB and ΔSO_4357-4358) were constructed by in-frame gene deletion mutagenesis. The IO₃⁻ reduction activity of the six mtr deletion mutants was tested in batch liquid cultures containing defined minimal medium amended with either formate or lactate as electron donor and IO₃⁻ as anaerobic electron acceptor. Results: The mtr deletion mutants ΔmtrA and ΔmtrB were unable to reduce IO₃⁻ with lactate as electron donor, but retained wild-type IO₃⁻ reduction activity with formate as electron donor. This result indicated that one of mtr paralogs may be required for IO₃⁻ reduction with formate as electron donor. Among the three mtr paralog mutants (ΔmtrA-ΔmtrDEF, ΔmtrA-ΔdmsEF and ΔmtrA-ΔSO_4360), only ΔmtrA-ΔdmsEF mutant displayed a deficiency in IO₃⁻ reduction activity with formate as electron donor. Subsequent IO₃⁻ reduction activity tests of three mtr paralog mutants (ΔmtrF, ΔdmsB and ΔSO_4357-4358) showed that ΔdmsB was unable to reduce IO₃⁻ while ΔmtrF and ΔSO_4357-4358 retained wild-type IO₃⁻ reduction activity.

Conclusions: Together these results provide complementary genetic and phenotypic evidence that the S. oneidensis dimethyl sulfoxide (DMSO) reductase displays broad substrate specificity and reduces IO₃⁻ as an alternative terminal electron acceptor.
Abstract Body:
Background: Contamination of soil and surface water bodies with toxic heavy metals due to various anthropogenic activities (such as urbanization and industrialization) remains a significant environmental concern. Among them, chromate (Cr(VI)), a mutagenic and carcinogenic pollutant, is being released in abundance in industrial effluents because of its uses in tannery, chrome plating, metal cleaning and other industries. In addition, this environmental problem has worsened in developing countries like Pakistan due to lack of proper legislation and monitoring system. Cr+6 is highly toxic as it can easily pass through cellular membranes, has high mobility, can interact with cellular proteins and nucleic acids. However, Cr+6 can be bio-converted into more stable, less toxic and insoluble trivalent chromium using microbes since bioremediation is highly economical and ecofriendly compared to other methods. Hence in this study, we have made efforts to utilize chromium tolerant bacteria for bio-reduction of Cr+6 to Cr+3. Methodology: For this, chromium resistant bacterial strain K1 was isolated from metal polluted industrial effluent from Kala Shah Kaku city, an industrial hub of Punjab-Pakistan. This bacterium exhibited maximum tolerance to hexavalent chromium up to 22mM Cr6+ which was further characterized by VITEK® 2 system and 16S rRNA gene sequencing. Other factors affecting the reduction of chromium such as initial chromate ion concentration, pH, temperature and contact time were also investigated. Moreover, functional groups of bacterial cell wall interacting with metal ions were also identified through Fourier-transform infrared spectroscopy (FTIR) and Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX) analysis. Results: On the basis of biochemical and phylogenetic analyses, We found that strain K1 was Staphylococcus aureus a Gram positive bacterium exhibiting optimum growth at 35°C, (pH =8.0). Under optimum conditions, it could remove about 80% chromium (Cr6+) after 16 hours with an initial metal concentration of 100 mg L−1. Data regarding SEM-EDX analyses confirmed enlargement of cells with irregular surface in presence of chromium. FTIR results assumed that carboxyl, amino and phosphate groups of cell wall were involved in complexation with chromium. Conclusions: Our results suggested that Staphylococcus aureus K1 is a novel; alternative and promising candidate that can be applied to remove chromium from metal polluted environments.
Session Title: AES06 - Bioremediation, Biodegradation, Biofouling, and Biocorrosion II
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/10/2018 2:45:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 5401
Poster Board Number: SUNDAY - 856

Abstract Title:
Targeting Microbial Arsenic Resistance Genes: A Meta-Analysis Across Soils

Primary Author Block:
T. K. Dunivin1, S. Yeh2, A. Shade1; 1Michigan State Univ., East Lansing, MI, 2Univ. of Maryland, College Park, MD

Abstract Body:
Arsenic is a ubiquitous and toxic metalloid, and its biogeochemical cycling is impacted by microbial metabolism. Despite extensive culture-dependent studies, the biogeography and diversity of arsenic resistance genes is under-characterized. We developed an open-access pipeline for testing different sequencing datasets for nine arsenic resistance genes: acr3, aioA, arsB, arsC (grx), arsC (trx), arsD, arsM, arrA, and arxA. Our pipeline includes BLAST databases, hidden markov model searches and gene-targeted assembly. Using this pipeline, we examined the distribution and diversity of arsenic resistance genes in soil. We first tested all genomes in the RefSoil database for arsenic resistance genes. 85.14% of all RefSoil genomes tested positive for at least one arsenic resistance gene. The most common genes detected were acr3, arsB, arsC (grx), and arsC (trx). To further investigate the diversity and abundance of arsenic resistance genes in soil, we used a gene-targeted assembler to test 39 public soil metagenomes from the USA, Malaysia, Russia, Brazil, and Canada for arsenic resistance genes. Coverage-adjusted abundance for each gene was normalized to single-copy gene rplB for comparison. Arsenic resistance genes were present in all soils tested; however, differences in genetic potential for arsenic metabolism was observed between sites. Thus, despite the ubiquity of microbial arsenic resistance genes, microbial communities differ in their potential to impact arsenic biogeochemical cycling. Furthermore, phylogenetic analysis revealed novel diversity of dissimilatory arsenic metabolism genes (aioA, arrA, arxA). Thus, our pipeline not only allows the biomonitoring of arsenic resistance genes but also has the potential to extend the known diversity of arsenic resistance genes. Future work that uses this pipeline will offer a more complete understanding of the diversity and occurrence of arsenic resistance genes, which ultimately will provide insights into the ecology of microbial arsenic resistance.
Detection of Sulfate Reducing Bacteria in Oil Produce Water and Biocide Treatment of Desulfovibrio

Primary Author Block:
I. Porat, V. Turk; Kemira, Atlanta, GA

Abstract Body:
Background: Oil production by water injection often leads to growth of sulfate-reducing bacteria (SRB) in reservoirs. Growth of SRB has many negative effects for the oil industry, including souring of oil by produced sulfide and corrosion of pipelines and storage tanks. Biocides are in use to inhibit the growth of SRB. Traditionally, the oil industry relies on cultivation techniques, like MPN liquid dilution series, to quantify SRB. The MPN method was accepted as the standard technique by the National Association of Corrosion Engineers (NACE) Standard TM0194-2004. Due to the long incubation time for the growth of the SRB using the MPN method (28 days) and the low microbial detection (less than 10% of the viable microbes could be cultured) molecular microbiology methods (MMMs) for the detection of SRB were included in the NACE Standard TM0212-2012. qPCR is one the MMMs method for detection and quantification of SRB, by proving the dsr genes (encoding the key enzyme in dissimilatory sulfate reduction pathway). In this research, we detected SRB in produced water samples by MPN and qPCR methods. In addition, biocides commonly used in oil and gas industry were tested against a strain of SRB.

Methods: Produce water samples, collected over 5 months, were used for detection of SRB by MPN (in Postgate media) or by qPCR targeting the dsr gene. Desulfovibrio vulgaris ATCC-29579 was grown in tubes with ATCC media 1249. Biocides effects were tested against the SRB model organism D. vulgaris.

Results: SRB were detected in all produced water samples, by qPCR, in the range of 2x10⁴ - 1x10⁶ gene copy number/ml. SRB were detected also by MPN, but in the range of 9x10⁰ - 3x10³ bacteria/ml. In most of the cases, the same trends over time were observed within the two methods.

Tetrakis(hydroxymethyl) phosphonium sulfate (or THPS, at 200, 300 and 400 ppm), glutaraldehyde-quaternary ammonium compounds (at 150, 300 and 500 ppm), dazomet (at 350 and 480 ppm), glutaraldehyde (at 500 ppm) inhibited the growth of D. vulgaris for up 33 days.

Conclusions: qPCR provides higher detection resolution, and in some cases, predicts the bacteria trends before the results were observed by the MPN method. As a result, the use of qPCR would detect a SRB spike faster and timely alert the facility to revise the biocide treatment.
Session Title: AES06 - Bioremediation, Biodegradation, Biofouling, and Biocorrosion II
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/10/2018 2:45:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 5713
Poster Board Number: SUNDAY - 858

Abstract Title:
Comparative Study of the Wild and Mutated Heavy Metal Resistant Klebsiella Variicola Generated for Lead Bioremediation

Primary Author Block:
Y. M. Feruke-Bello, O. Odeyemi, G. O. Babalola; Obafemi Awolowo Univ., Ile-ife, Nigeria

Abstract Body:
The decontamination of lead (Pb2+) contaminated soil is necessary due to its effects on soil microbial system, crop production and human health through the food chain. This research aimed at comparing the removal rate of Pb2+ from simulated contaminated soil by mutated and wild type of Klebsiella variicola. The soil samples were collected from Obafemi Awolowo University Teaching and Research Farm (OAUT&RF), air-dried, sieved, sterilized and spiked with 500 mg/kg of lead nitrate; the sterile and unsterilized soils were used as controls. The soil samples were thoroughly mixed and stored at 4oC for four weeks to establish equilibrium between the Pb2+ and soil, after which they were inoculated with the test bacteria and incubated for a period of 28 days. The comparison of the whole treatments revealed that total bacterial population peaked on the 14th day except in some few cases where the peaked was observed on the 7th and 21st day respectively. In the soils amended with Pb2+, highest total bacterial population of 8.24 ± 0.02 log cfu / g was observed by Klebsiella variicola MutEb on the 14th day, as compared to 8.16 ± 0.01 log cfu / g recorded for Klebsiella variicola Wt. As shown by atomic absorption spectrophotometer (AAS) analysis, Klebsiella variicola MutAc, Klebsiella variicola MutEb and Klebsiella variicola Wt removed 12, 9 and 4% of Pb2+ respectively from the simulated contaminated soils. In conclusion, artificially mutated bacteria appeared to offer greater potentials for use in remediating soil.
Abstract:

A Comparison of the Impacts of Biocides Glutaraldehyde and 2-2-Dibromo-3-Nitrilopropionamide on Aquatic Microbial Community Structure and Degradation Potential

Primary Author Block:
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Abstract Body:
Hydraulic fracturing (HF) is a method of hydrocarbon extraction that employs a mixture of pressurized water, chemicals, and sand to create fractures in hydrocarbon rich shale. Large volumes of hyper-saline fluids, chemicals, and hydrocarbons flow back to the surface. Potential spills of this wastewater are of environmental concern. Biocides are one of the most toxic chemicals used in HF. Biocides are added to prevent biocorrosion of equipment and gas souring. To understand the effect different biocides have on aquatic microbial community structure and degradation potential after a HF spill, two sets of microcosms were constructed using stream water impacted (HF+) and not impacted (HF-) by Marcellus shale HF operations. Microcosms were incubated aerobically for 56 days. One set of microcosms was spiked with glutaraldehyde (GA) and the second with 2-2-dibromo-3-nitrilopropionamide (DBNPA). These are the two most commonly used biocides in HF operations. Microbial community response to the biocides was monitored every two weeks using 16S rRNA amplicon sequencing. Biotic and abiotic GA and DBNPA degradation were measured using Ultra Performance Liquid Chromatography-Mass Spectrometry and High Performance Liquid Chromatography respectively. GA and DBNPA caused different responses in microbial community structure and degradation potential. GA HF+ microcosms experienced a smaller log fold reduction in 16S rRNA gene copies/mL than HF- microcosms. In contrast, HF- DBNPA microcosms experienced a smaller log reduction, but by day 56 HF+ was more enriched than HF-. This could be attributed to DBNPA biocidal activity depleting over time. Additionally, more members of the microbial community of HF+ were able to tolerate GA and DBNPA as shown by higher alpha diversity. Beta diversity and differential expression analysis for sequence count data showed different bacterial enrichment between HF+ and HF- microcosms after addition of the biocides. However, the phylogenetically distinct response between HF+ and HF- microcosms was different between GA and DBNPA. Furthermore, less taxa was differentially enriched in DBNPA than in GA. Biotic degradation was faster in HF- than HF+ microcosms for both GA and DBNPA. Meanwhile abiotic degradation was minimal for GA while DBNPA was easily degraded. Overall, GA caused a more pronounced microbial response and was more persistent over time. These findings demonstrated a lasting effect on microbial community structure and suppressed degradation potential in streams impacted by HF operations.
Abstract Title:
Expression of Fungal Cutinases in Pichia Pastoris for Polyesters Degradation
Primary Author Block:
L. Vazquez Alcántara, R. Oliart Ros, C. Peña Montes; Natl. Technological of Mexico (TNM), Inst. Tecnológico de Veracruz, Veracruz, Mexico
Abstract Body:
Polyester degradation is an important application of fungal cutinases. Cutinases are commonly secreted by fungi and bacteria, being the most studied the plant pathogenic fungi. The purpose of the present work was to identify phytopathogenic fungi that degrade polyesters which are contained in plastic residues. Two important fungi were identified from 12 isolated fungi. They were identified by ITS sequencing as Alternaria alternata and Moniliophtora roreri. We have isolated the cutinase-encoding genes reported in the genome of this pathogenic fungi and expressed in the yeast Pichia pastoris. Genomes of fungi were obtained from NCBI. Each genome was analyzed to found cutinase sequences using the software Multaline and with the Expasy translate tool was obtained the protein translation. For the desing of the primers was employed the software OligoAnalyzer Tool. RT-PCR amplifications (six genes) were accomplished using Pfu DNA Polymerase system. RT-PCR products were sequencing. Sequences were analyzed with the software MEGA 6 and then a phylogenetic tree were elaborated. To transform P. pastoris, vector pPICZ containing cutinase gene, was linearized by digestion with Pmel and then introduced into P. pastoris X-33 by electroporation. The 3 sequences of cutinases of A. alternata presented signal peptide and two sequences of three cutinase genes of M. roreri presented signal peptide. The primers designed for the six cutinases where successfully applied for the amplification by PCR with the cDNA obtained from fungi grown in cutinase induction medium. The sequences obtained were compared with the sequences already reported in the NCBI, showing similarities between 98 and 99%. A phylogenetic tree containing the 6 sequences of cutinases with cutinases reported degrading biopolymers was made. The cutinases of M. roreri remained in a clade next to the clade where cutinases of Fusarium langsethiae are grouped. The 3 cutinases of A. alternata are found in different clades. For example, the cut1 of A. alternata is grouped in the same branch of a cutinase of A. nidulans. P. pastoris was transformed with the linearized vector pPICZ containing each cutinase gene previously cloned in E. coli DH5α. Clones were grown on YPDS agar medium containing Zeocin and a polyester (polycaprolactone or polystirene) for selection. Clones with a transparent halo were selected as positive. They were verified by PCR using cutinase specific primers. We could obtain 6 clones, each one containing a different fungal cutinase capable of hydrolyze polyesters.
Role of the Megaplasmid Pswit02 in Dioxin & Dibenzofuran Degradation by Sphingomonas Wittichii Rw1

Primary Author Block:
S. S. Eleya, G. Zylstra; Rutgers Univ., New Brunswick, NJ

Abstract Body:
Sphingomonas wittichii RW1 metabolizes dioxin and dibenzofuran as sole carbon and energy sources. Sphingomonas wittichii RW1 is considered a model organism that facilitates the study of the molecular and biochemical mechanisms of dioxin and other environmental toxicants metabolism. The complete genome sequence of RW1 reveals that this strain, in addition to a main chromosome contains two circular megaplasmids pSWIT01 and pSWIT02. A number of studies have suggested that pSWIT02 carries genes that are involved in dioxin and dibenzofuran degradation. However, recent work in our laboratory has shown that the ring cleavage dioxygenase (second enzymatic step) and hydrolase (third enzymatic step) of the dioxin catabolic pathway are encoded by the chromosome. It is our hypothesis that the megaplasmid pSWIT02 only encodes the first enzymatic step of the dioxin pathway and we aim in this study to better clarify the role of pSWIT02 in Sphingomonas wittichii RW1. We constructed a new plasmid (pSEZ_RW1) containing the origin of replication oriR and associated partition (par) genes of pSWIT02 along with genes for tetracycline resistance. This plasmid was mated into RW1 with the goal of forcing out the megaplasmid pSWIT02 since the shared oriR and par would result in incompatible plasmids. The dual plasmid construct was subcultured multiple times in LB liquid medium and colonies were examined for the presence of pSWIT02 by PCR. This eventually resulted in a pSWIT02 cured version of RW1. The pSEZ_RW1 plasmid is slightly unstable and loss of this plasmid was then obtained by selecting for colonies lacking tetracycline resistance. Unsurprisingly, the pSWIT02 cured strain did not grow on either dioxin or dibenzofuran. We used PCR to clone the dxnA1A2, fdx3, and redA2 genes encoding a multicomponent angular dioxygenase from three different locations in pSWIT02 into the low copy number vector pRK415 so that expression of the genes is from the lac promoter. Moving this plasmid into the cured RW1 restored growth on dioxin and dibenzofuran. Growth curves on minimal medium supplemented with either compound as the sole carbon source showed that the rate and extent of growth was almost the same as the wild type strain. Based on these experiments we conclude that the only pSWIT02 genes involved in the degradation of dioxin and dibenzofuran are the ones encoding the initial angular dioxygenase. This explains why very few dioxin degrading organisms are known, that a combination of plasmid and chromosome encoded genes are necessary for growth on this recalcitrant compound.
Abstract Title:
Culture-Dependent Approaches to Isolate and Cultivate Hydrocarbon-Degrading Bacteria
Primary Author Block:
T. N. Jackson, A. Hyun, Y-F. Lu; Westmont Coll., Santa Barbara, CA
Abstract Body:
Studying marine microorganisms in a laboratory setting has produced innovation in the pharmaceutical and biotechnological fields. Yet, with most bacterial cultivation methods today, only a small percentage of bacteria from a source is isolated and cultured. We hypothesized that a culture-dependent optimization of media and laboratory growth conditions for marine microorganisms would enrich the isolation and cultivation of specific types of microorganisms. We tested a combination of isolation methods and culture conditions and identified a suitable approach to cultivate hydrocarbon-degrading bacteria. Marine bacterial samples obtained from Butterfly Beach, Santa Barbara, CA were incubated in different laboratory environments with respect to the lighting, temperature, agar media recipes, and salt concentration of the saline during purification. The 16S rRNA sequencing and comparison of the bacterial samples to the entries in the Ribosomal Database Project (RDP) indicated that aquatic bacteria isolated with 1) the saline concentration identical to the ocean during the purification procedures, 2) cultured with nutrient agar plates made with the equivalent osmotic pressure, salinity, and mineral content as sea water, and 3) incubated with access to daylight at approximately 20ºC, produced morphologically diverse colonies. All identified strains belonged to the class Gammaproteobacteria, which includes the genera Pseudoalteromonas, Shewanella, Psychromonas, and Marinobacter. This is a class known to degrade petroleum and is only in high abundance following an oil spill. This finding suggests that the method described above may be considered as a viable method to isolate, cultivate, and study marine hydrocarbon-degrading bacteria species.
Abstract Title:
Unveiling A New Pathway for Microcystin Degradation by Bacteria

Primary Author Block:
A. Krishnan, X. Mou, M. Gangoda; Kent State Univ., kent, OH

Abstract Body:
Background: Excessive nutrient loading in the form of industrial waste and fertilizer run off has led to the formation of harmful cyanobacterial blooms (CyanoHABs) in Lake Erie. CyanoHABs produce a number of cyanotoxins including a class of liver toxins called microcystins (MCs). In natural environments, MCs are primarily removed through microbial activities. Degradative pathway adopted by MC-degrading bacteria was first identified in members of Sphingomonas as an mlr gene based, step-wise cleavage process. However, a recent metagenomics study has suggested the presence of an alternative pathway for MC degradation in Lake Erie. Methods: To identify this novel pathway, MC-degrading bacteria were isolated from Lake Erie and confirmed not carrying mlr genes. One of these bacterial isolate (LEw-24) was further analyzed by transposon mutagenesis to obtain mutants that lost MC degradation capabilities. Results: The genomic regions flanking the mutation was sequenced and identified using Sanger sequencing. The putative functional sequences were assigned as unidentified proteins based on BLAST analysis. To identify intermediates during the degradation, aliquots of LEw-24 cell extract and MC-LR mixtures were taken at 2 hr intervals and analyzed by LC-MS. Conclusion: A new pathway was proposed based on the LC-MS results, which was different from the known mlr-based pathway. Keywords: Microcystins, transposon, mutagenesis, intermediates
Session Number: 431
Session Type: Poster

Session Title: AES06 - Bioremediation, Biodegradation, Biofouling, and Biocorrosion II
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/10/2018 2:45:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 6900
Poster Board Number: SUNDAY - 864

Abstract Title:
Rhodococcus Sp. Nsx2 Modulates Trace Metal Phytoremediation Efficiency by Reshaping Rhizosphere Microbiomes

Primary Author Block:
J. Hou, W. Liu, L. Wu; Inst. of Soil Sci., Nanjing, China

Abstract Body:
Background: Trace metal contamination is a growing problem in agriculture soils. Application of hyperaccumulator inoculated with beneficial bacteria to extract trace metals contaminated soils seems a promising strategy. However, the mechanisms underlying plant-microbe interaction during this process, especially at the whole bacterial community level, remain elusive.

Methods: A pot experiment was conducted to study the impact of the bacterial inoculation on the indigenous bacterial community and their corporate effects on the phytoremediation efficiency. Cd/Zn hyperaccumulator Sedum Plumbizincicola was planted with/without plant beneficial bacterial strain Rhodoccus sp. NSX2. Then, highthroughput sequencing and RMT-based network analysis was used to track bacterial community in both the rhizosphere and the bulk soil in the two treatments.

Results: NSX2 did enhance the phytoremediation efficiency in this study. Also, NSX2 was found to survive in the rhizosphere with high relative abundance of about 1.46% and to reshape the whole bacterial community structure in the rhizosphere. Moreover, NSX2 made the bacterial network in the rhizosphere more complex through a positive cooperation strategy with the indigenous bacteria while the network in the bulk soil unchanged.

Conclusions: We concluded that bacterial inoculation enhanced phytoremediation efficiency not only depended on the inoculated beneficial bacteria itself but also on the subsequently changed indigenous bacterial community at a whole level.
Session Title: AES06 - Bioremediation, Biodegradation, Biofouling, and Biocorrosion II
Session Start Date Time: 6/10/2018 12:45:00 PM
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Session Primary Track: Applied and Environmental Science
Abstract Control Number: 7095
Poster Board Number: SUNDAY - 865

Abstract Title:
Evaluation of the Diffusion Chamber and Microbial Trap Approach to Isolate Uranium and Nickel Resistant Soil-Borne Biodegradative Microorganisms

Primary Author Block:
R. Jaswal, A. Pathak, B. Edwards, III, D. Chappell, A. Chauhan; Florida A&M University, Tallahassee, FL

Abstract Body:
Microorganisms underpin most of the biogeochemical transformations in the environment including remediation of toxic contaminants. However, less than 1% of microbes can be cultivated and isolated under standard lab conditions. Towards this end, we evaluated the use of diffusion chamber (DC), and microbial trap (MT) approach for the cultivation and isolation of Uranium (U) and Nickel (Ni) resistant bacteria from historically contaminated environments. Soils were collected from the Steed Pond location at the Savannah River Site (SRS), SC which remains co-contaminated by radionuclides, metals and organics from the nuclear era weapon production activities. Briefly, soils are diluted with sterile water, combined with sterile agar, inoculated into a chamber that is lined by membrane filters and placed on wet soils; this permits the diffusion of nutrients into the chamber and facilitates growth of environmental microbiota within the agar. We established DC with the membrane pore size of 0.03 µm on both sides of the chamber. Simultaneously, MT were also established such that the top membrane was of a pore size of 0.03 µm and the bottom membrane, which is in contact with the soils, is of 0.2 µm size. In the MT, soil actinomycetes enter the agar through the bottom 0.2 µm size membrane and are ‘trapped’ in the chamber. After incubating for 20 days, colonized biomass from within the agar plugs were spread onto LB amended with U and Ni to isolate and identify potentially novel biodegradative microbes. A part of the biomass was also transferred into new plugs and processed for 2-3 cycles; biomass after each cycle was serially diluted and plated onto LB with U and/or Ni for microbial enumeration. 16S gene based identification of the DC and MT isolated strains revealed Burkholderia sp. to be the most abundant U and Ni resistant microbiota. Also obtained were two Penicillium strains using only the MT approach, which were previously not isolated by the traditional culturing. Interestingly, amplicon based metagenomic analysis also confirmed that Burkholderia spp., and Penicillium spp., were the most abundant bacterial and fungal genera in the soils used for establishing the diffusion chambers. Statistical analysis indicated that the microbial diversity was considerably different between the DC and MT approaches when compared to plate cultures, suggesting that this approach can be successfully applied to isolate metal resistant microbiota which can then be studied for their environmental remediation abilities.
Abstract Title:
The Influence Of in situ Activated Carbon on Biodegradation of Chlorinated Solvents
Primary Author Block:
K. McGee; Clemson Univ., Clemson, SC
Abstract Body:
A technology has emerged in the last two years for dissolved plume control at chlorinated solvent contaminated sites; it is referred to as in situ activated carbon amendment. Activated carbon is an adsorption technology that is well known and has been used ex situ for decades. It is primarily marketed by two major vendors, although there are several smaller market vendors that also provide the material. The vendors claim it readily adsorbs contaminants, removing them from the aqueous medium, then facilitates long-term biological and chemical degradation processes that transform the contaminants to innocuous end products. There are two critical reasons for investigating this remediation technology. First, it has been readily adopted by regulators so it is incumbent upon the research community to investigate this technology so it is applied in the most productive manner. Second, and more importantly, there are no peer reviewed data indicating that the strategy works as marketed—that microbial activity will degrade contaminants that sorb to activated carbon in situ. We are using both powdered activated carbon (PAC) and granular activated carbon (GAC) in TCE-contaminated aquifer material to determine how pre-adsorption of TCE influences secondary microbial and chemical reactions. Early data suggests that TCE is not completely reduced to ethene at stoichiometric levels in any incubations. In EOS amended incubation when PAC is added in at low loading mass and no carbon mass, TCE is also reduced to ethene. However, the concentration of ethene present is not stoichiometric relative to the initial concentration of TCE added, suggesting that the sorbed TCE may not yet be fully available for microbial respiration. In all other activated carbon amended systems, TCE has yet to be reduced to ethene. The daughter products cis-dichloroethylene (cis-DCE) and vinyl chloride (VC) are present to varying amounts in all incubations, but are generally higher in those without activated carbon. Thus far the data suggest that activated carbon limits secondary microbial activity, but we will run experiments long-term to determine if sorbed TCE will be reduced. Future data will include quantifying genes relevant to complete dechlorination such as bvcA, vcrA, tceA, and Dehalococcoides specific 16S rRNA genes. In addition, all residual TCE will be extracted from the activated carbon to determine the extent of contaminant still sorbed despite ongoing microbial activity.
Abstract Title:
Phenotypic Characterization and Ecological Succession of Microorganisms During the Fermentation of Cassava and Maize

Primary Author Block:

Abstract Body:
Background: Fermented foods are consumed by a very large population in Africa but the products have many drawbacks ranging from shelf life instability to contamination and toxicity. These foods therefore require an upgrade through improved fermentation processes. This work determined the phenotypic characteristics of the fermenting microorganisms and microbial ecological succession during fermentation of cassava and maize to determine the predominant fermenting microorganisms.

Methods: Cassava roots and maize grains were fermented using the traditional method of processing them into fufu and ogi for 72 h and 48 h respectively. Samples were drawn every 12 h for analysis. Enumeration and characterization of lactic acid bacteria were carried out on MRS medium with subsequent microscopic examination, physiological, biochemical reaction tests and API 50 CH gallery. Yeast isolates were identified by their morphological characteristics. Results: Thirteen lactic acid bacteria were isolated from the fermenting cassava and 6 from the fermenting maize. The Isolates were Gram positive and catalase negative. Lactobacillus plantarum, L. fermentum and L. pentusus predominated in both fermentations while Candida tropicalis, C. krusei and Saccharomyces cerevisiae also predominated in both fermentations. Candida inconspicuo was found only in cassava fermentation. Conclusion: The results of this work revealed the microbial ecology of fermented cassava and maize which is a prerequisite to the understanding needed to develop a multifunctional starter culture for these fermentations for their upgrade.
Abstract Title:
Response Surface Methodology for Enhanced Cmcase Production by Newly Isolated Strain B. Licheniformis Tlw-3
Primary Author Block:
T. Kiran1, W. Asad1, M. Ajaz2, S. A. Rasool1; 1Univ. of Karachi, Karachi, Pakistan, 2Federal urdu Univ. of Arts, Sci. and Technology (FUUAST), Karachi, Pakistan
Abstract Body:
Cellulases have gained global attention because of their varied applications in multiple industrial setups such as paper and pulp, food and biotechnology, agricultural, pharmaceutical, wine and brewery, textile and detergents industries. The current era of cellulase application is the bioconversion of agro-industrial wastes into valuable (output) products such as xylose, glucose, and arabinose which are then fermented to ethanol ["biofuel" (economy driver of a country)]. Cellulase is a multienzyme system contained various enzymes with different isozymes that work synergistically for complete hydrolysis of cellulosic biomass. Enzyme production is critically influenced by various factors such as incubation temperature, initial pH of the medium, cultivation time, and nitrogen-carbon sources. Multiple parameters for optimization constitute a lengthy and time-consuming process, therefore, RSM can be applied to study the significance of multiple interactions of several factors on enzyme production. In this regard, response surface methodology was performed using a central compost design (CCD) constructed by Minitab software 17. Six variables (previously optimized by one factor at a time approach) i.e. pH, temperature (°C), incubation time (hour), the concentration of yeast extract, peptone and carboxymethyl cellulose (CMC) were taken. A set of 53 experiments was designed. These experiments were conducted in a 100ml flask containing 25ml of sterilized medium and incubated at varied conditions according to the design. Afterwards, the cell-free extract was collected by cold centrifugation at 1148xg for 20min. Enzyme activity was estimated by DNS method. The data was then analyzed in Minitab 17. Data was found significant and the model was fit with 0.11 lack of error which shows the fitness of the model. Contour and response surface plots showed the good interactions among the factors. The optimized response was found at pH 6.6, temperature 70 °C, peptone 18.1 g/l, yeast extract 14.01 g/l, CMC 16.11 g/l and incubation period of 62 hours.
**Abstract Title:**
Production of Multienzyme Cocktail from Yeast Consortium Under Solid State Fermentation of Sugarcane Bagasse

**Primary Author Block:**
M. Shariq; Univ. of Karachi, Karachi, Pakistan

**Abstract Body:**
The sustainable utilization of (lignocellulosic) LC substrates for biorefineries necessitates exploring indigenous microbial strains capable of utilizing locally available substrates. Pakistan being a major producer of sugarcane, generates huge quantities of sugarcane bagasse (SB). Being rich in carbohydrates, SB offers tremendous potential to be utilized in bio-based processes. At present, the material is underutilized and the country does not have established fermentation industries. Being recalcitrant in nature, SB is not readily utilized by all types of microorganisms, however, a large number of fungal and bacterial strains have been reported for the utilization of SB. Where as, very few yeasts strains have been described in this regard. The present study was aimed at the development of yeast consortium for the effective utilization of SB with the co-production of three industrially important plant cell wall degrading enzymes (CWDE) i.e. Cellulase, Xylanase and Pectinase. Three strains, MK-157, MK-178 and AA-15 were selected based on the previous reports for their ability to produce, cellulase, xylanase and pectinase, respectively. Solid state fermentation was carried out using SB as substrate and mineral salt solution as moistening agent. The strains were inoculated in different combinations and at different time intervals. Crude enzyme preparations were obtained after adding buffer to post-incubation SB, followed by centrifugation. Crude preparation was assayed for cellulase, xylanase and pectinase activity. The results depicted that the consortium produced higher titers of pectinase, however, the titers of cellulase and xylanase were not increased significantly. The simultaneous production of three different CWDE in appreciable titers renders the significance of the developed consortium. Further studies are required to investigate the suitability of the multienzyme preparation for the saccharification of agro-wastes.
**Abstract Title:**
Evaluation of Xylanolytic Activity from An Indigenously Isolated Candida Tropicalis Yeast Strain

**Primary Author Block:**
M. Shariq; Univ. of Karachi, Karachi, Pakistan

**Abstract Body:**
Xylanase (Xyl) is the group of enzymes that catalyze the hydrolysis of 1, 4-β-D-Xylosidic linkages in Xylan, the most abundant hemicelluloses moiety in plant cell-wall. Bacteria and molds are preferred source for Xyl, whereas, yeasts have scarcely been reported for their xylanolytic potential. In the present study, out of 225 indigenous yeast strains, 84 strains elaborated Xyl in xylan supplemented agar medium amongst which 40 strains yielded higher titers (>0.2 IU ml-1) in broth medium. The parameters for Xyl production from the most promising strain, MK-160, were optimized as 40oC temperature, pH 7 and 0.5% substrate concentration. The Xyl preparation from MK-160 displayed optimal activity at acidic pH and 40°C in presence of 2% Xylan. The strain was identified on the basis of morphological and cultural characteristics as Candida tropicalis MK-160. It showed the ability to ferment sugarcane bagasse-and wheat bran with the concomitant production of higher titers of Xyl and low levels of endoglucanase (EG). The strain was able to absorb more than 96% of congo red from solution and to produce 5.45% ethanol in glucose supplemented medium. The data showed that the strain can further be evaluated for its potential to use for waste management and some other biotechnological processes.
Abstract Title:
Immobilization of β-1,4-Xylanase from Thermomyces Lanuginosus C9 Isolated From Hot Spring

Primary Author Block:
S. Khan1, J. Kiran1, M. Irfan2, F. Hasan1, A. A. Shah1; 1Quaid-i-Azam Univ., Islamabad, Pakistan, 2Sarhad Univ. of Sci. and Information Technology, Peshawar, Pakistan

Abstract Body:
Background: The stability as well as re-usability of enzyme at high temperature, narrow pH range, metal ions, organic solvents, surfactants and salts is the main issue. Enzyme immobilization is an exciting alternative to improve the stability as well as its reusability in various industrial processes. Methods: β-1,4-xylanase producing fungal strain was isolated from hot spring and identified through morphological as well as rDNA gene sequencing. Different physico-chemical conditions were statistically optimized using Placket-Burman design software for maximum xylanase production. The enzyme was purified to homogeneity by column chromatography and its molecular size was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The purified enzyme was characterized as well as immobilized in agar-agar matrix for investigating its thermostability as well as re-usability. Results: The fungal strain C9 was grouped into genus Thermomyces with 99% similarity to Thermomyces lanuginosus strain RMB (KF207598). The molecular size of purified β-1,4-xylanase was determined approximately 23 kDa. The specific activity of β-1,4-xylanase was 201.31 U/mg with 8.93-fold purification and 62.53% yield. The enzyme was active in the presence of various metal ions except Cd+2 and Hg+2 that strongly inhibited its activity. Surfactants such as Tween 20, Tween 40, Tween 60, Tween 80 and Triton X-100 potentially enhanced xylanase activity upto 1% concentration. The enzyme was stable in the presence of 15% organic solvents (Methanol, formaldehyde and glycerol) for 120 minutes. The purified xylanase was immobilized in agar-agar matrix and activity was monitored with respect to the free enzyme. Maximum immobilization yield was achieved at 4% concentration with an increase in optimal reaction time from 05 to 25 min and recycling efficiency approximately upto 7 cycles. The enzyme displayed high thermal stability in immobilized state, and retained 100% of its initial activity at 70oC up to 150 min in comparison to free enzyme that lost 16% of its activity at this temperature. The Km and Vmax of both free and immobilized xylanase was 4.19 mgml−1 and 5.32mgml−1 and 235.78 and 50.25 μmolmg−1min−1, respectively. Conclusions: The resistance to organic solvent, metal ions and detergents as well as stability to high temperature and broad pH range represents its efficiency for various biotechnological applications under extreme conditions.
Abstract Title:
Optimisation of Protease Production in Indigenous Bacillus Spp. Isolated from Soil Samples in Lagos, Nigeria Using Response Surface Methodology

Primary Author Block:
Y. L. Suberu; Univ. of Lagos, Lagos, Nigeria

Abstract Body:
Proteases catalyse the hydrolysis of peptide bonds in proteins and offer a huge potential for application in industries, including detergent, dairy, leather, baking, pharmaceutical and beverage industries. In this study, indigenous Bacillus spp were isolated from soil samples collected from abattoir, refuse and non-refuse sites in Lagos, Nigeria and optimized for protease production. The isolates were purified on Bacillus agar and screened for protease production on casein agar. Three strains showing high potential for protease production were identified as Bacillus cereus ABBA1, Bacillus subtilis RD7 and Bacillus subtilis NRD9 via amplification and analysis of 16S rRNA genes. Protease optimization was done insilco using Box-Behnken Design (BBD) by response surface methodology (RSM) with Design-Expert software and then validated experimentally. Factors optimized include temperature, pH, carbon source, nitrogen source and inoculum volume. Statistical analysis was done using ANOVA. The results obtained from the Insilco experimental model revealed high protease activity of 171.10 U/ml, 136.76 U/ml and 133.76 U/ml while experimental validation generated a high protease activity of 200.03 U/ml, 176.00 U/ml and 163.76 U/ml for strains ABBA1, RD7 and NRD9, respectively in optimized medium. This corresponds to 33.54-, 42.21- and 36.64- fold increase in protease production compared to the unoptimized protease production medium. The optimum conditions for extracellular protease production obtained from quadratic model of RSM were 40°C, pH 8.5, 2.5% (v/v) inoculum volume, 1.5 g/L maltose and 2.0 g/L beef extract powder. The model prediction agreed with the experimental data (R² = 0.98) and was statistically significant (p < 0.05). Results from this study further confirms the need to optimize the production parameters to achieve maximum yield and economical use of available resources during production of industrially important enzymes.
Abstract Title:
Glutaminase-Free L-Asparaginase Production by Leucosporidium Muscorum Isolated from Antarctic Marine-Sediments

Primary Author Block:
A. Pessoa Junior1, R. Barros Freire1, R. Bertelli Ferraro1, L. Sette2, F. Lourenço1; 1Sch. of Pharmaceutical Sci., Univ. of São Paulo, sao paulo, Brazil, 2Inst. of BioSci.s, Statal Paulista Univ. “Julio de Mesquita Filho”, sao paulo, Brazil

Abstract Body:
L-asparaginase (ASNase) is an important therapeutic agent used in the treatment of acute lymphoblastic leukemia (ALL). Bacterial ASNase preparations available on the market have some limitations related to their low stability in serum, which lead to severe immunological reactions in 3-78% of patients. Yeasts are highlighted as important microorganisms for ASNase production since they are able of producing similar enzymes to human congeners and with fewer side effects. In this work, the screening of 145 yeasts isolated from marine-sediments in King George Island, Antarctica, resulted in nine glutaminase-free L-asparaginase producing yeasts. A strain of Leucosporidium muscorum was the isolated that yielded the highest ASNase activity (490.41 U.L-1) and volumetric productivity (5.12 U.L-1.h-1). Carbon and nitrogen sources were evaluated by a method of variation in one factor at a time. Sucrose, yeast extract and proline resulted in maximal production of enzyme and were selected for Czapek Dox Medium (CDM) optimization by full factorial design. Optimum media condition for yeast growth and ASNase yield were 20 g.L-1 sucrose, 15 g.L-1 yeast extract and 20 g.L-1 proline and resulted in 4,582.5 U.L-1 of enzyme and 63.64 U.L-1.h-1 of volumetric productivity. This is the first report on production of ASNase by a cold-adapted yeast which may indicate a new potential source of glutaminase-free L-asparaginase for commercial purpose.
Abstract Title:
Cellulase Production from Penicillium Citrinum Using Pineapple Peels As A Cheap, Alternate Substrate

Primary Author Block:
O. Oyedeji; Obafemi Awolowo Univ., Ile-Ife, Nigeria, Ile-Ife, Nigeria

Abstract Body:
Cellulases are a major group of enzymes having wide range of industrial and biotechnological applications. The production cost of cellulase is a major factor limiting its use hence the need to develop low cost production systems for this enzyme. In this study, brewer’s spent grain and pineapple peels, which otherwise constitute sources of pollution to the environment, were investigated as cheap, alternate substrates for cellulase production from Penicillium citrinum, isolated from decaying orange fruits. Cellulase production was assayed by measuring the amount of glucose released in µmole per millilitre per minute by using the dinitro salicylic acid (DNS) method. Evaluation of process parameters affecting cellulase production was carried out. Cellulase titres 3.82 ± 0.136 U/ml and 1.405 ± 0.151 U/ml were produced by the fungus, using pineapple peels and brewer’s spent grain as substrates, respectively, under submerged fermentation. Maximum cellulase production from P. citrinum occurred with the use of pineapple peels as substrate, at 72 h fermentation period, with the use of pineapple peels at a concentration of 1.5%w/v and peptone as the best nitrogen source. The optimum temperature for the production of cellulase by the fungus was found to be 50 oC while the optimum pH was 6.0. Findings from this study indicated the potential use of pineapple peels as cheaper alternative substrate for the production of cellulase thus mitigating the hazardous effect it has on the environment as pollutant. P. citrinum was able to grow and produce good levels of cellulase using solely pineapple peels as low cost substrate, at high temperature of 50 oC, making this strain and this low cost agro-industrial residue worthy of further investigation and potentially feasible for wide-range of biotechnological applications.
Abstract Title:
Isolation of Tetragenococcus Halophilus Strains Showing Proteolytic Activities from Myeolchi Jeotgal (Anchovy Jeotgal), A Korean Fermented Sea Food
Primary Author Block:
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Abstract Body:
Myeolchi jeotgal (Anchovy jeotgal) is a traditional Korean salted and fermented sea food prepared by mixing myeolchi and salt. We prepared myeolchi jeotgal (NaCl 23%, w/v) and fermented for 24 weeks at 15°C. Lactic acid bacteria (LAB) were isolated from 4 and 12 weeks fermented jeotgal. One hundred ninety nine LAB were isolated from MRS agar plates containing 0.006% bromo cresol purple and 10% NaCl. Twelve strains showed proteolytic activities on tryptic soy agar plates with skim milk (1%, w/v) and NaCl (5%, w/v). They were identified as Tetragenococcus halophilus, based on 16S rRNA gene and recA gene sequences. They grew well at 15% NaCl (w/v) and grew slowly at 20% NaCl (w/v), but did not grow at 25% NaCl (w/v). Based on acid, neutral and alkaline activities, 4 strains (BS1-37, BS2-36, SS3-2 and PS1-25) were selected for further studies. Three strains (BS1-37, BS2-36 and PS1-25) grew well at initial pH of 6-9 and grew slowly at pH 10. SS3-2 grew well at pH 6-8 and grew slowly at pH 9, but did not grow at pH 10. When their growth at different temperature (4, 15, 20, 30, 37 and 42°C) was tested for 45 days, all strains grew well at 20 and 30°C and the OD600 values reached to 1.0-1.2. They grew slowly at 15°C, and the OD600 values reached to 0.5-0.7. The viable counts of BS1-37, BS2-36, SS3-2, and PS1-25 grown for 15 days at 15°C were 4.06 × 10⁷, 6.51 × 10⁷, 8.40 × 10⁷, and 6.03 × 10⁷ CFU/ml, respectively. Considering their high salt tolerance and growth at 15°C, these Tetragenococcus strains seem useful as starters for jeotgal or fish sauces.
Optimization of Chitinase Production B&It the Antarctic Bacterium Arthrobacter Ps&Itchrochitiniphilus Strain 492
Primary Author Block:
Y. M. Santa Cruz Vasquez1, A. W. Fernandes Duarte2, V. Maia de Oliveira1; 1Campinas State Univ., Campinas, Brazil, 2Federal Univ. of Alagoas, Arapiraca, Brazil
Abstract Body:
Background: Chitin is the most abundant polymer in nature, distributed widely in marine and terrestrial environments. In Antarctica, this polymer is mainly associated to marine invertebrates. Chitinase acts in the hydrolysis of the β-1-4 bond of N-acetyl-D-glucosamine and may used for different biotechnological applications. The aim of this study was to optimize the production of cold-active extracellular chitinase from Arthrobacter psychrochitiniphilus strain 492 isolated from Antarctica. Methods: This isolate was recovered from biofilm soil collected during the expedition OPERANTAR XXXII (summer 2013/2014) in Half Moon Island after cultivation in R2A culture medium and incubation at 15°C. For optimization of chitinase production, the effects of nutrient composition (chitin, peptone, extract yeast, K2HPO4, KH2PO4, MgSO4.7H2O, NH4NO3, and NaCl) and physicochemical parameters (pH) were evaluated using Plackett and Burman (PB) method, followed by 25-1 fractional factorial design and finally a Central Composite Design (CCD 23). Chitinase activity was enhanced by the interaction between chitin (2.0 - 5.5%) and NH4NO3 (1.5 - 5.0 g/L). Results: The optimization of culture conditions with the aid of mathematical modeling increased 7.8-fold the enzyme activity when the maximum activity of CCD 23 (222,73 U/mL) was compared with that obtained with the culture medium without optimization (28,80 U/mL). Conclusions: Statistical experimental design was an efficient tool in the optimization of chitinase production by the Antarctic A. psychrochitiniphilus 492 bacterium and this enzyme may be employed in biotechnological processes at low and moderate temperature. Keywords: psychrophilic bacteria, Bioprospecting, Low Temperature
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**Session Primary Track:** Applied and Environmental Science

**Abstract Control Number:** 6768  
**Poster Board Number:** SUNDAY - 878

**Abstract Title:**  
Production of Polygalacturonase Using Watermelon Rind by Semi-Solid Fermentation

**Primary Author Block:**  
S. E. Omonigho, S. A. Ogbebor; Univ. of Benin, Benin City, Benin City, Nigeria

**Abstract Body:**  
Background: Pectic substances are the complex polysaccharides present in the middle lamella of plant and are degraded by a group of enzymes called pectinases. These enzymes are pectin esterase, pectin lyase and polygalacturonase. Most commercial preparations of pectic enzymes are obtained from fungal sources using agricultural wastes such as wheat bran, citrus peel and orange bagasse. This study was aimed at producing polygalacturonase from watermelon rind using fungi isolated from decaying watermelon by semi-solid fermentation.

Methods: Different fungal species were isolated from decaying watermelon using Sabouraud Dextrose Agar with pour plate technique. The fungal isolates were then sub-cultured on pectin agar plates and screened for pectinolytic activity as indicated by the diameter of clear hydrolysed zone. Three (03) fungal isolates with good potentials of pectinolytic activity were selected for the production of polygalacturonase using watermelon rind as major substrate. The reducing sugars in crude extracts were determined using the dinitrosalicylic acid (DNS) method and the concentration of protein was determined using Lowry’s method.

Results: Five (05) fungal species were isolated, culturally and morphologically characterised, and identified namely; species, Aspergillus niger, species of Fusarium, Mucor, Penicillium and Saccharomyces. All the fungal isolates depolymerised citrus pectin on the plate assay with clear zones varying from 5.00 to 8.00 cm in diameter. Aspergillus niger, Fusarium species and Penicillium species were selected for polygalacturonase production using watermelon rind as substrate. After 7 days of fermentation, Penicillium species, showed maximum enzyme activity (23.62 ± 1.30 U/ml), followed by Aspergillus niger (18.04 ± 0.56 U/ml) and Fusarium species (13.21 ± 0.19 U/ml). The highest protein concentrations were recorded on day 4 of fermentation for Aspergillus niger (31.25± 1.25 mg) and Penicillium species (19.38± 0.62 mg) and on day 7 for Fusarium species (15.00± 1.25 mg).

Conclusions: Findings from this study have revealed that pectinolytic fungi isolated from spoilt watermelon are good candidates for polygalacturonase production. Watermelon rind is a suitable low-cost substrate for the production of polygalacturonase and its utilisation will help to reduce pollution load in the environment.
Abstract Title:
Process and Metabolic Engineering to Enhance Furfural Tolerance by

Primary Author Block:
C. V. Agu, V. Ujor, T. C. Ezeji; Ohio State Univ., Wooster, OH

Abstract Body:
A major challenge to efficient bioconversion of lignocellulosic biomass hydrolysates (LBH) to fuels and chemicals stems from the toxicity posed by lignocellulose-derived microbial inhibitory compounds (LDMICs). The reducing equivalent, (NAD[P]H), is a vital metabolic tool that microorganisms deploy during production of alcohols such as ethanol and butanol as well as detoxification of LDMICs during fermentation of LBH. Since glycerol catabolism to pyruvate generates twice as much NAD[P]H as sugar (e.g. glucose and xylose) catabolism, we reasoned that concomitant utilization of glycerol and glucose by Clostridium beijerinckii would enhance solvent (acetone-butanol-ethanol: ABE) production in the presence of LDMICs. However, inefficient utilization of glycerol by C. beijerinckii is a major impediment to adopting glycerol metabolism as a strategy to increase NAD(P)H regeneration, alleviate LDMICs (e.g., furfural) toxicity, and improve fermentation of LBH to butanol. To address this bottleneck, we employed a metabolic engineering strategy to enhance glycerol utilization by C. beijerinckii. By overexpressing two glycerol dehydrogenase (Gldh) genes (dhaD1 and gldA1) from the hyper-glycerol utilizing C. pasteurianum as a fusion protein in C. beijerinckii, we achieved approximately 42% increase in glycerol consumption when compared to the plasmid control. Further, C. beijerinckii_dhaD1+gldA1 achieved a 59% increase in growth (in the presence of glycerol), while butanol concentration and ABE productivity increased by 14% and 55.6%, respectively, relative to the control (glucose-only medium). Co-expression of DhaD1+GldA1 and GldA1 + dihydroxyacetone kinase (DhaK) resulted in significant payoffs in cell growth and ABE production when compared to expression of one Gldh. With 4 to 6 g/L furfural challenge, increased glycerol consumption by DhaD1+GldA1 strain increased cell growth (>50%), the rate of furfural detoxification (up by 68%), and ABE concentration (up by 40%) relative to the plasmid control. Likewise, over-expression of DhaD1+GldA1 + DhaK improved butanol and ABE production by 70% and 50%, respectively in the presence of 5 and 6 g/L furfural relative to the plasmid control. Taken together, overexpressing glycerol dehydrogenases from C. pasteurianum in C. beijerinckii significantly enhanced glycerol utilization, ABE production, and furfural tolerance by C. beijerinckii.
Abstract Title:
Statistical Optimization of Endoglucanase Production by A Thermophilic Strain of Bacillus Licheniformis Rt-17

Primary Author Block:
R. Tariq, I. Ansari, F. Qadir, A. Ahmed, M. Shariq, U. Zafar, A. Ahmad, S. Khan, M. Sohail; Univ. of Karachi, Karachi, Pakistan

Abstract Body:
Thermostable cellulases are required for a variety of commercial processes. Bacillus is a house of thermostable proteins. Screening of indigenously isolated strains of bacteria revealed the promising production of cellulase by a strain, RT-17, at 50°C. The strain was identified on the basis of biochemical and molecular characteristics as B. licheniformis. The factors affecting cellulase production from B. licheniformis RT-17 were evaluated for their significant effect using Plackett Burman Design and were optimized by employing Box-Behnken Design. The model predicted 9.808 IU/ml of endoglucanase (EG) under optimum conditions of 50°C; 10% inoculum size; pH 5; and 1% peptone in fermentation medium. Practically, a titer of 9.128 IU/ml was obtained, showed the validity of the model. The enzyme preparation from B. licheniformis RT-17 was applied in combination with xylanase and pectinase preparations from indigenous yeasts for the hydrolysis of sugarcane bagasse (SCB). A higher degree of synergy (7.1 folds) was observed when yeast pectinase was used with bacterial cellulase for the hydrolysis of alkali treated SCB. Whereas, the degree of synergy was lower when bacterial cellulase was mixed with yeast xylanase. The study revealed the possibility of utilization of combination of yeast and bacterial enzymes for biomass saccharification.
Abstract Title:
Optimization of Some Parameters in the Culture Medium of Candida Guilliermondii and Issatchenkia Orientalis for Lysine Production
Primary Author Block:
C. C. Ekwealor, L. O. Chike-Mozie, I. A. Ekwealor; Nnamdi Azikiwe Univ., Awka, Nigeria

Abstract Body:
Background: L-Lysine is an essential amino acid required in diets of man and animals. It is important for growth and maintenance of the body. It is used in medicine for preventing and treating cold sores. Bacteria are known to produce lysine but not much is known about lysine production by yeasts. The aim of this work is to examine the influence of some cultural parameters on lysine production by Candida guilliermondii and Issatchenkia orientalis. Methods: The effects of sucrose, fructose, arabinose, glycerol and glucose at 20.0% (w/v) and NH4Cl, KNO3, urea and (NH4)2SO4 at 10.0% (w/v) in a fermentation medium for lysine production by Candida guilliermondii and Issatchenkia orientalis were investigated. The influence of fermentation medium volume (20.0, 30.0, 35.0, 40.0ml) in a 100ml Erlenmeyer flask and inoculum size (1.0, 1.5, 2.0, 2.5ml), on lysine production by the yeasts were examined. The effects of varying concentrations of arabinose (20.0, 40.0, 60.0, 80.0g/l) and (NH4)2SO4 (10.0, 20.0, 30.0, 40.0, 60.0g/l) on lysine production by the two organisms were determined. Results: Arabinose and (NH4)2SO4 were observed to be carbon and nitrogen sources of choice respectively, for lysine production by the yeasts. Medium volume of 20ml and inoculum size of 2.5ml for Candida guilliermondii and 1.5ml for Issatchenkia orientalis enhanced lysine production. Lysine levels of 2.40mg/ml and 2.68mg/ml were accumulated in the fermentation medium by Candida guilliermondii and Issatchenkia orientalis. Arabinose at 80.0%(w/v) and (NH4)2SO4 at 40.0%(w/v) stimulated lysine levels of 3.63mg/ml and 3.48mg/ml in the broth cultures of Candida guilliermondii and Issatchenkia orientalis respectively.
Conclusions: Optimization of some cultural parameters was observed to improve lysine accumulation by Candida guilliermondii and Issatchenkia orientalis.
Abstract Title:
Global Transcriptomic Response of Modified Bacillus anthracis over Expressing Protective Antigen

Primary Author Block:
A. K. Sharma, S. H. Leppla, J. Shiloach; NIH (NIH), Bethesda, MD

Abstract Body:
Background: Native protective antigen (PA) expressed by Bacillus anthracis has been used as a vaccine against anthrax infections in humans. However, its expression as recombinant protein in other microorganisms were often associated with inclusion body formation, and endotoxin contamination. Therefore, expression of the recombinant PA from its native producer B.anthracis is being considered as preferred production approach. Methods: The presented study focuses on understanding the effect of over expression of recombinant PA on the metabolism of the host producer B.anthracis ames strain (BH500). The study was conducted by growing the bacteria in a bioreactor and by comparing the expression of the bacterial genes between the non-producing and the rPA producing strain. With the expectation that the information obtained can contribute to improve PA and other recombinant proteins from Bacillus. Results: Comparative RNAseq analysis showed that in the culture, expressing rPA sigL, the gene encoding RNA polymerase σ54 was upregulated together with the general stress transcription factor sigB (fc ~3) and its regulators rsbW & rsbV, and the global regulatory repressor ctsR (fc 4.9). Contrary to the general observations of increase in heat shock chaperone transcripts at post recombinant protein expression stress, down-regulation of groL, groES, hsiO, narJ, hscC, dnaJ, dnaK, grpE, clpB, clpP was observed. In addition, most of the genes in PA expressing culture belonging to TCA, glycolysis, PPP, amino acid biosynthesis and transport of heavy metals and short peptides were up-regulated in the lag phase. Likely to help the cell to cope with the increased requirements for nutrient and energy required for PA expression. These pathways are down-regulated in the log and latelog phase as cellular stress response onsets, which is reflected in the decreased specific growth rate. Conclusions: The findings from this study indicate that global regulators such as sigL, ctsR, sigB play crucial role in controlling major metabolic pathways in B.anthracis ames strain and are probably responsible for decreased growth rate associated with the PA expression. The information obtained from this study would be further used for genetic modification of B. anthracis to improve PA production.
Session Title: Quantifying the Robustness of Ethanol Production by Crabtree Negative Yeasts: Dev. of A Method Based on the Principles of Growth on Complementary Substrates

Primary Author Block: S. Maitra, A. Narang; Indian Inst. of Technology Delhi, New Delhi, India

Abstract Body:
It is widely believed that the ethanol production by Crabtree negative yeasts is not robust. These yeasts undergo purely respiratory metabolism under aerobic conditions; on the other hand anaerobic conditions promote fermentation but discourage cell growth and viability (1, 2). This has led to the hypothesis that growth and fermentation occur only under very restricted conditions. However, this lack of robustness has never been rigorously defined or studied. In this work, we have used Scheffersomyces (Pichia) stipitis as representative yeast to characterize the robustness of ethanol production in Crabtree negative yeasts. Our method for characterizing robustness is based on the principle of growth on complementary substrates i.e. substrates that satisfy entirely different nutrient requirements (3). Specifically, we show that if a chemostat operating at a fixed dilution rate (D) and oxygen mass transfer coefficient (kla) is fed with progressively increasing glucose feed concentrations (sf), the culture passes through three distinct regimes: (i) At low sf, the culture is carbon limited, i.e. oxygen is in excess and there is no ethanol production. (ii) At high sf, the culture is oxygen limited, i.e. glucose is in excess and there is ethanol production. (iii) At intermediate glucose feed concentrations, both glucose and oxygen are limiting, and ethanol is produced without loss of unused glucose from the chemostat. We define robustness as the width of the dual-limiting regime, i.e., the range of the glucose feed concentrations supporting ethanol production without loss of glucose. Additional experiments show that the robustness of ethanol production varies with D and kla. However, this variation can be captured by a simple unstructured model, which predicts all the operating conditions that ensure robust ethanol production, i.e., ethanol production without loss of glucose in the effluent.
Modulation of the Microbial Composition During In Vitro Fermentation of Alcohol Insoluble Solids Derived from Indonesian Local Sweet Potato (Ipomoea Batatas) by A Human Fecal Inoculum: Effects of Bacto-Pepton and Casein Supplementation

Z. H. Hassan, Erwin G. Zoetendal, Henk A. Schols, Hauke Smidt; Wageningen Univ., Wageningen, Netherlands

Background: Undigested fibre and protein are among the macronutrients in diet most associated with fermentation. Both are not absorbed in the small intestine, but are differently fermented by colonic microbiota, hence differently affect the microbial composition and metabolic activity. The objective of this study was to evaluate the effects of nitrogen of bacto-pepton and casein supplementation on the microbiota composition and metabolite production during an in vitro fermentation of Indonesian local sweet potato (Ipomoea batatas)-derived alcohol insoluble solids (AIS) by human fecal inoculum.

Methods: DNA was extracted from the fermentation digests using the repeated bead beating method and the microbial community composition was analysed by Illumina HiSeq sequencing of 16S ribosomal RNA gene fragments amplified with primer pair of 515F-806R targeting the V4 region. The main metabolite products, i.e. gases (hydrogen, methane, and carbon dioxide), short chain fatty acids (acetate, propionate, and butyrate), lactate, and ammonium was measured throughout the fermentation. Galacto-oligosaccharides (GOS) were used as positive control, and media without added carbon source were used as blank control. Results: Results showed that upon the fermentation of sweet potato-derived AIS, the microbial diversity both in the presence of bacto-pepton and casein (SP+N) and absence of bacto-pepton and casein (SP-N) dropped during the first 6 hours after which it started to stabilize. However, the decrease was much higher in the SP+N. After 48 h, Senegalimassilia, Mogibacterium, Blautia, Coprococcus 3 and Dorea that were present quite abundant in SP+N were not found in SP-N. Furthermore, an obvious distinction on the metabolite production was observed. Carbon dioxide, acetate, propionate, butyrate, lactate, and ammonium were produced substantially higher in SP+N compared to SP-N. Conclusions: In conclusion, supplementation of nitrogen of bacto-pepton and casein during the fermentation of Indonesian local sweet potato-derived AIS shifted the microbial composition, decreased the microbial diversity but increased the major final products of fibre fermentation, which suggesting a switch from fibre fermentation to protein fermentation.
Abstract Title:
Optimization of Fermentation Conditions for the Biosynthesis of Dextran by Weissella Cibaria Cmgdex3

Primary Author Block:
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Abstract Body:
Background: W. cibaria CMGDEX 3, isolated from cabbage was previously reported by us (Ahmed and Siddiqui,2012) to produce water soluble high molecular weight dextran of >200,000 Daltons dextran with predominant α (1→6) glycosidic linkages Methods: Weissella cibaria CMG DEX3 a potent dextran producer was previously isolated from cabbage, structure and physical characteristics of produced dextran were studied in detail by us (Ahmed and Siddiqui, 2012). Production of Dextran by CMGDEX 3 was carried out in medium containing g/l of sucrose 150, bactopeptone 5, yeast extract 5, K2HP04 15, MnCl2 0.01, NaCl 0.01, CaCl2 0.05 and pH was adjusted to 7. Dextran production, extraction and purification were done by ethanol precipitation method (Ahmed and Siddiqui, 2012). To study the effect of temperature on dextran synthesis ,incubation temperatures were varied from 15°C to 45ºC (with the difference of 5ºC) while other culturing conditions were kept constant. pH of media was adjusted to different ranges from 5-10 before autoclaving while other culturing conditions were kept constant. The effect of sucrose concentration on dextran production was studied by varying its concentration from 5 to 25% (with the difference of 5%). Other culturing conditions were kept constant.Effect of incubation time on yield of dextran was studied at different time intervals 6, 12, 18, 24, 30 and 36 hrs. Results: Optimized temperature observed for maximum yield of crude dextran from W. cibaria CMGDEX 3 was 25°C. Optimum pH of the growth medium, for the highest yield of dextran (105g/l) was found to be 8.0 while second highest yield (91g/l) was obtained at pH 9 but dextran synthesized at these pH became partially soluble in water. However at pH 7 and 7.5 yield of dextran was 76g/l and 79g/l, respectively and the synthesized dextran was completely soluble in water. Optimization for sucrose concentration resulted in maximum yield of crude dextran (84g/l) at 20% sucrose in the medium whereas second highest production of dextran (81g/l) was observed at 15% sucrose concentration. Production of crude dextran by W. cibaria CMGDEX3 increased with incubation time at optimized culturing conditions which reached maximum(78g/l) at 24 hrs. Conclusions: In the present study W. cibaria CMGDEX3 appeared as a promising producer of high yield of crude dextran and these optimized culturing conditions could be used for the commercial production of dextran for various industrial applications.
Abstract Title:
Effects of Vitamins, Amino Acids, Antibiotics and Bivalent Metals on Methionine Production by Bacillus pumilus and Bacillus amyloliquefaciens

Primary Author Block:
I. A. Ekwealor, T. M. C. Asogwa; Nnamdi Azikiwe Univ., Awka, Nigeria

Abstract Body:
Background: Methionine, a nutritionally essential amino acids, is indispensible for all animal species. It is used in animal feed for livestock production and as flavour in food additives. The use of synthetically produced methionine has been limited in many countries hence the need for ecromethionine based on natural resources. This paper aims at investigating the effects of vitamins, amino acids, antibiotics and bivalent metals on methionine accumulation by Bacillus pumilus and Bacillus amyloliquefaciens

Methods: Medium for methionine production by B. pumilus and B. amyloliquefaciens contains 8.0% glucose and 4.0% as carbon and nitrogen sources respectively. The effects of varying concentrations (0.1, 1.0, 10.0, 100.0 µg/ml) of vitamins: nicotinic acid, riboflavin, thiamine, vitamin B12 and of bivalent metals: on Methionine accumulation by the bacterial organisms were studied. Varying concentrations (0.001, 0.01, 0.01, 1.0 µg/ml) of antibiotics: Ampicillin, Cloramphenicol, Ciprofloxacin, Tinidazole, and 0.01% of amino acids: lysine, alanine, glycine, glutamine, cysteine, DL-leucine were examined for their effects on methionine production by the organisms. Results: All vitamins increased methionine production by B.pumilus while vitamin B12 and riboflavin improved methionine accumulation by B. amyloliquefaciens. All bivalent metals enhanced methionine yields by B. amyloliquefaciens but had no effect on B.pumilus. All concentrations of Ampicillin stimulated methionine accumulation by B. pumilus, however, Chloramphenicol (0.01µg/ml) produced a methionine level of 7.01mg/ml. Tinidazole at all concentrations increased methionine production by B. amyloliquefaciens. All amino acids except glycine improved methionine accumulation by B. amyloliquefaciens while alanine and glycine stimulated methionine production by B. pumilus. Conclusion: It was observed that methionine yields by B.pumilus and B.amyloliquefaciens can be stimulated by vitamins, amino acids and antibiotics.
Abstract Title:
Engineered Biocatalysts for Synthesis of Fucosyl Oligosaccharides
Primary Author Block:
R. Chen; Georgia Inst. of Technology, Atlanta, GA
Abstract Body:
Microbial catalysis has recently emerged as one of the most promising approaches in oligosaccharide synthesis. However, despite significant progresses, microbial synthesis still requires much improvement in efficiency and in reduction of process complexity. Additionally, in light of the stunning natural diversity and many varied applications, broadening the range of glycans accessible via microbial synthesis is of paramount importance to many scientific fields and medical, food, diagnostic applications. Major challenges in microbial synthesis include catabolite repression and high cellular energy requirement. Here we demonstrated a new approach to overcome these challenges by directly tapping into the cellular “power house”, the TCA cycle, to sustain biocatalysis. This approach not only circumvents catabolite repression but also eliminates acidic glycolysis byproducts. As such, the whole-cell biocatalysis can be carried out without sophisticated fed-batch feeding and pH control. The system could achieve several grams per liter (3-4 g/L) within a 24 hours period in shaker flask cultivation for three targets, fucosyllactose, fucosyllactulose, demonstrating both high efficiency and great versatility of the biocatalyst developed. To the best of our knowledge, this is the first use of respiration for oligosaccharide synthesis and the first description of successful synthesis of fucosyllactulose.
Abstract Title:
Development of A High Efficient Transformation Sys. and the Production of Long-Chain Dicarboxylic Acids in Candida Sorbophila Ds02

Primary Author Block:
H. Lee, C. Han, J. Ahn, H. Lee; Korea Res. Inst. of BioSci. and Biotechnology, Cheongju-si, Korea, Republic of

Abstract Body:
Background: Long-chain dicarboxylic acids (LDCAs) are multipurpose chemicals widely used in polymers, perfumes, plasticizers, lubricants, and adhesives. The biotransformation of LDCAs from alkanes and fatty acids by microorganisms has attracted recent interest, since synthesis via chemical oxidation causes problems in terms of the environment and safety. Especially, alkane assimilation yeasts mainly used as a host for the conversion because of their strong ω-oxidation. Here, we isolated ω-oxidizing yeast from petrochemical factory using chemostat enrichment culture. In addition, for the development of LDCA producing mutant, we constructed efficient transformation tool and disrupted POX genes encoding Acyl-coA isozymes in Candida sorbophila DS02. Methods: For the development of LDCA producing mutant, chemostat enrichment culture, antibiotic susceptibility test, flow cytometry analysis, site-directed transformation, and fed-batch fermentation were performed. Results: After 168 hours of cultivation, one strain was finally isolated from chemostat enrichment culture, which was named C. sorbophila DS02. As a result of the antibiotics susceptibility test, complete inhibition of the growth was observed on YPD plates containing 600 µg/ml hygromycin B, 200 µg/ml noruseothricin and 300 µg/ml phleomycin, respectively. As a result of the flow cytometry analysis, it was identified to have haploid life cycle. And also, it was confirmed that arrested cells in S phase had higher site-specific targeting efficiency than cells in early-log and stationary phase. Finally, POX deleted mutant was developed, which produced 20g/l of dodecanedioic acid for 48 hours in fed-batch fermentation. Conclusions: According to be increased interest in the microbial conversion of LDCAs from alkanes and fatty acids as an alternative to chemical synthesis, it is one of the major challenge to screen and develop microorganisms. Therefore, we isolated an emerging yeast with high ω-oxidation ability and provided the basic metabolic engineering tools for use as a biotechnological platform for biochemical production.
Abstract Title:
Improved Search Method for Methionine-Producing Actinomycetes in Soil
Primary Author Block:
C. C. Ekwealor, N. N. Uchefuna, I. A. Ekwealor; Nnamdi Azikiwe Univ., Awka, Nigeria
Abstract Body:
Background: Actinomycetes represent an ubiquitous group of microbes widely distributed in natural ecosystem around the world. They exhibit diverse physiological and metabolic properties such as production of extracellular enzymes and other bioactive molecules of importance in medicine, industry and agriculture. Not much is known about their involvement in amino-acid production. The objective of this paper is to develop an improved search method for isolating methionine-producing actinomycetes.

Methods: Actinomycetes were isolated from soil samples on Starch-Casein agar medium and the recovered isolates screened for methionine-production by streaking them on the surface of solid agar medium seeded with methionine-auxotroph, Escherichia coli. The plates were observed for halo growths of E. coli after 72h incubation at 30°C. Methionine production in a shake flask fermentation medium containing 0.4% (w/v), glucose and 0.2%(w/v), (NH4)2SO4 by methionine-producing isolates were examined. The very active methionine producer was then characterized. Results: A total of 96 isolates were recovered from different soil samples. Halo growth of the auxotroph, E. coli, on the surface of the solid agar medium was an indication of methionine production by the isolates. Seven of the methionine-producers recovered accumulated methionine levels in the fermentation medium, which were observed to be proportional to the halo growths of the E. coli on solid agar medium. The very active methionine producer was identified as Streptomyces badius. Conclusions: The improved search method developed for isolating methionine-producing actinomycetes is a simple and fast screening process.
Abstract Title:
Bioconversion of Potential Efficacy Compound in Medicinal Plants Through Lactic Acid Bacteria Isolated from Kimchi

Primary Author Block:
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2Korea Univ. of Sci. and Technology, Daejeon, Korea, Republic of

Abstract Body:
The purpose of this study was to develop functional foods as a multi-effect anti-diabetic agent that work simultaneously on various targets through microbial conversion of compounds in medicinal plants. For the screening of strains with bioconversion activity, 280 strains of lactic acid bacteria (35 species in 5 genera including 2 novel species) were isolated from a traditional Korean fermented vegetable, kimchi. Among them, bioconversion was confirmed in the Lactobacillus spp., Enterococcus sp., Pediococcus sp., and Leuconostoc sp. through the submerged fermentation with medicinal plants. Representatively, Lactobacillus spp. or Enterococcus sp. demonstrated that initially abundant geniposide and gastrodin in the medicinal plants are converted into its corresponding aglycones, genipin and 4-hydroxylbenzyl alcohol, respectively, after the fermentation at 28 °C for 48 h without additional nutrient supplementation. Substances present as glycosides in medicinal plants can only be effective after metabolism through intestinal microorganisms. From this perspective, our results also confirm that the efficacy of substances in medicinal plants have increased through microbial bioconversion. The anti-diabetic effect of bio-converted compounds will be discussed in detail in this text.
Phenyllactic Acid Production by Recombinant Escherichia coli from Sorghum Bagasse: Fermentation Inhibition by the Enzymatic Hydrolysate

Primary Author Block:
H. Kawaguchi1, K. Yoshihara1, T. Hasunuma1, T. Sazuka2, N. Takaya3, C. Ogino1, A. Kondo1; 1Kobe Univ., Kobe, Japan, 2Nagoya Univ., Nagoya, Japan, 3Univ. of Tsukuba, Ibaraki, Japan

Abstract Body:
Phenyllactic acid (PhLA) is a promising building block for bio-based materials, as PhLA can be polymerized into the biopolymer poly-PhLA. A drought-tolerant C4 grass, Sorghum (Sorghum bicolor L. Moench), requires less fertilizer and water, and has similar annual productivity per acre, as compared with other energy crops. Thus, dilute acid-pretreated sorghum bagasse can serve as a suitable lignocellulosic feedstock for the microbial fermentation, since the fibrous plant material remaining after juice extraction was predominantly composed of glucan (59%) and xylose (7.2%). However, fermentation inhibition was observed when sorghum bagasse hydrolysate was used as carbon source for PhLA production by recombinant Escherichia coli (Kawaguchi, et al. 2015). To investigate the fermentation inhibition for PhLA production, we compared the contents of potential fermentation inhibitors released from sorghum bagasse during enzymatic hydrolysis using two sorghum cultivars and the inhibitory effects of potential inhibitors on PhLA fermentation were examined. Dilute acid-pretreated sorghum bagasse was prepared from stems of the hybrid sorghum cultivar Tentaka and a brown midrib (bmr) mutant bmr-6 which reduces lignin content, and a recombinant Escherichia coli strain GK1 expressing phenylpyruvate reductase from Wickerhamia fluorescens was used for PhLA production from enzymatic hydrolysate of the sorghum bagasse as a carbon source. As compared to bmr-6, pretreated Tentaka bagasse was more recalcitrant for enzymatic hydrolysis with cellulase cocktail, Cellic CTec2 (Novozymes), and reduced PhLA production from the enzymatic hydrolysate by 50% (400 mg/L). The contents of potential fermentation inhibitors in the two hydrolysates before PhLA fermentation were determined by GC-MS. Tentaka bagasse hydrolysate contained more syringaldehyde and phenolic acids (ferulic, p-coumaric, and syringic acids) than bmr-6 hydrolysate. Addition of p-coumaric acid in fermentation culture reduced PhLA titer in a dose-dependent manner. Taken together, these findings suggest that p-coumaric acid serves as a fermentation inhibitor of PhLA fermentation with sorghum bagasse hydrolysate, particular in Tentaka hydrolysate, although such an inhibitory effect has not been reported for E. coli.
Exploring Haloarchaea As A Cell Factory for Bioplastic Production

Primary Author Block:
H. Xiang; Inst. of Microbiol., Chinese Academy of Sci., Beijing, China

Abstract Body:
The halophilic archaeon Haloferax mediterranei, has shown promise for the production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV), a desirable bioplastic, from many low-cost carbon sources. It undergoes cell lysis and releases the PHA granules in distilled water, providing an important strategy to reduce the PHBHV production costs. To obtain a full understanding and to engineer H. mediterranei as an efficient cell factory for PHBHV biosynthesis, we have sequenced its genome and developed the gene knock-out system for this halophile. A genome-wide investigation of the PHBHV biosynthesis in this haloarchaea was performed by bioinformatic analysis, gene knockout/complementation, as well as 13C metabolic flux analysis. These approaches have revealed the key genes for PHBHV biosynthesis (phaA, phaB, bktB, phaE and phaC) and granule modulation (phaR and phaP), the pathways for precursors (e.g. propionyl-CoA) supplying and PHBHV mobilization (phaR and phaZ) as well as its linkage to acetyl-CoA and propionyl-CoA assimilation. These advances have also revealed many haloarchaeal-specific enzymes and pathways in PHBHV biosynthesis, such as the archaeal-type PHA synthases (IIIA type), the haloarchaeal-type β-ketothiolases with two distinct subunits, and the novel 3-hydroxypropionate pathway coupling CO2 fixation into PHBHV. Interestingly, we found that knockout of the genes involved in exopolysaccharide biosynthesis, decrease of the metabolic flux for gluconeogenesis, or reducing the genome DNA copies will efficiently direct more matter and energy for PHBHV biosynthesis, thus enhanced PHBHV productivity by approximately 20-60% under a similar culture condition. Our works suggest that H. mediterranei is among the most important PHBHV producers. A comprehensive use of the available knowledge, including pathway engineering and optimization of the high-density fermentation with low-cost carbon sources, may result in the industrial-scale production of high-quality PHAs by H. mediterranei, thus providing a novel and efficient cell factory for bioplastic production in the near future.
Abstract Title:
Cloning, Expression and Structure-Function Relationship of Low-Temperature Active and Alkaline Stable Protease from Psychrotrophic Acinetobacter Sp. Ihb B 5011

Primary Author Block:
R. Salwan1, V. Sharma1, A. Gulati2, R. Kasana3; 1Chandigarh Univ., Gharuan, Chandigarh, India, 2CSIR-Inst. of Himalayan Bioresource Technology, Palampur, India, 3Central Arid Zone Res. Inst., Jodhpur, India

Abstract Body:
Exploration of psychrotrophic bacteria from the cold deserts of Lahaul and Spiti in the Indian Western Himalayas was undertaken for characterization of low temperature active and alkaline stable proteases of industrial importance. A total of 719 bacterial isolates were screened for protease activity from soil and sediment samples of Lahaul and Spiti, India. Protease activity was observed by 50 isolates at 5 °C and pH 10. The protease ~35 kDa was purified with 9.8 fold increase in specific activity of 6540 U mg⁻¹ protein. The purified enzyme was active over 4 to 60 °C and pH 7-11 with optimum at 40 °C and pH 9 and strongly inhibited by PMSF, indicating serine-type of protease. The enzyme was compatible retaining more than 50% activity with surfactants, oxidizing agent and commercial detergents, and effectively removed blood stains on cotton fabrics indicating its suitability as additive in laundry. Further, the gene encoding low temperature active and alkaline stable protease was amplified and revealed an ORF of 1323 bp encoding protein of 441 amino acids with predicted molecular weight 47.2 kDa. The deduced amino acid sequence consisted of N-terminal signal peptide 1-21 amino acids, propeptide 22-143 amino acids, peptidase S8 domain 144-434 amino acids, and C-terminal region 435-441 amino acids. The structural characteristics of high R/(R+K) ratio, more glycine and less proline residues, higher negatively charged amino acids (E+D), less salt bridges, and longer loops also appeared responsible for adaptations under alkaline conditions and low temperature. For expression of protease in E. coli BL21(DE3), constructs containing signal peptide pET-Alp, without signal peptide pET-Alp1 and with peptidase S8 domain pET-Alp2 were made and functionally active protease was recovered after refolding and maturation. The subtraction of pre-peptide from precursor protease Alp revealed the variation in protein size which was estimated 47.2 kDa based on nucleotide base pairs and ~35 kDa based on the amino acid residues. The characterization of recombinant protease from Acinetobacter sp. IHB B 5011 (MN12) revealed low temperature and alkaline nature of enzyme. The similarities in the properties of mature active recombinant protease expressed in E. coli to those of the native protease indicated the suitability of Alp as an attractive candidate for industrial applications.
Abstract Title:
Purification and Quantitation of A Vero-Cell Derived Rabies Virus Glycoprotein for Vaccine Formulation and its Correlation with In Vivo Potency Tests

Primary Author Block:
M. Almario, V. Rincón, A. Barbosa, F. Gonzalez, J. Ossa, J. Granados, Z. Suarez-Moreno; Empresa Colombiana de Productos Veterinarios Vecol S.A., Bogota, Colombia

Abstract Body:
Out of major structural proteins of the rabies virus, only glycoprotein (G-protein) is used as a reference to formulate rabies vaccines. Most vaccines are liquid suspensions of inactivated rabies virus, obtained from cell infected cultures followed by inactivation and purification. To assess the quality of vaccines batches before release, in vivo potency tests (NIH test) are used, which are performed on mice followed by a challenge test using an intracerebral injection of live rabies virus. In accordance with international regulations, vaccines are formulated with at least 1 IU of rabies glycoprotein (G-protein) per dose, which implies the use of quantitation methods to estimate the amount of glycoprotein produced during the manufacturing processing. The glycoprotein is usually measured using commercial ELISA kits, which are not validated and often show low correlation with the NIH potency test results. The scope of this study was to design an accurate method to quantitate Rabies G-protein, which could provide a suitable correlation to potency tests in order to increase G-protein yields and process efficiency. For this purpose, a high concentration stock of rabies-virus purified G-protein (RVGP) was obtained from a virus-infected VERO-cell culture by using a two-step process of preparative liquid chromatography with an AKTA pure instrument (GE Life sciences). This stock was later used to standardize an interferometric technique (BLItz® Pall Life sciences) that allows quantitating the RVGP with higher precision and sensitivity than commercial ELISA kits, in less than 30 minutes. Experiments performed on mice revealed an improved correlation between the interferometry technique and the potency test results, regarding other techniques. These results allowed us to monitor the amount of G-protein along vaccine production with increased accuracy. Furthermore, to comply with the three Rs (replace, reduce, and refine) in animal research, our project is looking forward to validating this interferometry assay as an alternative to other commercial ELISA kits to reduce the number of mice used to release each batch of vaccine. The technique developed with the BLItz® system does not require any secondary labels for the antigen and our results suggest that is more reliable than ELISA or western blot. Our results support the fact that the BLItz® system will be an ideal model for the quantification of viral antigens that can be extrapolated for any viral system and could be implemented as a routine test in the production and formulation of vaccines.
Abstract Title:
Novel Swollenin from Talaromyces leycettanus JCM12802 with Broad Substrate Specificity and Synergistic Action with A Cellulase on Avicel Degradation

Primary Author Block:
Y. Wang, F. Zheng, T. Tu, H. Luo, B. Yao; Feed Res. Inst., Chinese Academy of Agricultural Sci., Beijing, China

Abstract Body:
Background: Conversion of the crystalline regions of native cellulose into amorphous and accessible regions is a critical step, and is a great challenge for efficient hydrolysis of lignocelluloses. Fungal swollenin, which can disrupt avicel, have great potential for applications in conversion of biomass, food and animal feed industries. Methods: Swollenin gene, swo, was isolated from the thermophilic fungus Talaromyces leycettanus JCM12802 and successfully expressed in Pichia pastoris. The main degradation products were analyzed by HPAEC-PAD. Results: The purified recombinant TlSWO was optimally active at 50 °C and pH 3.0, and exhibited excellent stability over a broad pH range of 2.0-9.0, and was highly thermostable at 60°C and below. The TlSWO was highly active towards laminarin, CMC-Na, barley b-glucan, glucomannan and lichen polysaccharide. The main products degraded by TlSWO from cellotetraose, cellopentaose and cellohexose were cellobiose and cellotriose, orcellobiose, cellotriose and cellotetraose, respectively. TlSWO and cellulase from Novozymes showed significant synergistic effects on the degradation of avicel, releasing more reduced sugars by simultaneous addition.

Conclusions: This study provides a novel fungal swollen in with broad substrate specificity for synergistic degradation for the efficient hydrolysis of plant biomass.
Session Number: 433
Session Type: Poster
Session Number: 433
Session Type: Poster
Session Title: AES10 - Genetic and Metabolic Functions in Environmentally Relevant Microbes: Carving Out A Niche
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/10/2018 2:45:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 6649
Poster Board Number: SUNDAY - 899

Abstract Title:
Genomic Analysis OfArthrobacterSp. Srs-W-1-2016 Provides Insights on Niche Adaptation for Survival in Uraniferous Soils
Primary Author Block:
A. Pathak, R. Jaswal, B. Edwards, III, A. CHAUHAN; Florida A&M Univ., Tallahassee, FL
Abstract Body:
Arthrobacter sp. strain SRS-W-1-2016 was isolated from the Savannah River Site (SRS) that remains co-contaminated by radionuclides, heavy metals, and organics. SRS is located on the northeast bank of the Savannah River (South Carolina, USA), which is a U.S. Department of Energy (DOE) managed ecosystem left historically contaminated from decades of nuclear weapons production activities. Predominant contaminants within the impacted SRS environment include U and Nickel (Ni), both of which can be transformed microbially into less toxic forms via metal complexation mechanisms. Strain SRS-W-1-2016 was isolated from theuraniferous SRS soils on 4200 μM U, but rapid growth was observed at much lower concentrations of 500 μM U, respectively. Microcosm studies established with strain SRS-W-1-2016 revealed a rapid decline in the concentration of spiked U such that it was almost undetectable in the supernatant by 72 h of incubation. To obtain a deeper understanding of the metabolic potential, a draft genome sequence of strain SRS-W-1-2016 was obtained at a coverage of 90×, assembling into 93 contigs with an N50 contig length of 92,788 bases. The genomic size of strain SRS-W-1-2016 was found to be 4,564,701 bases with a total number of 4327 putative genes. An in-depth, genome-wide comparison between strain SRS-W-1-2016 and its four closest taxonomic relatives revealed 1159 distinct genes, representing 26.7% of its total genome; many associating with metal resistance proteins (e.g., for cadmium, cobalt, and zinc), transporter proteins, stress proteins, cytochromes, and drug resistance functions. Additionally, several gene homologues coding for resistance to metals were identified in the strain, such as outer membrane efflux pump proteins, peptide/nickel transport substrate and ATP-binding proteins, a high-affinity nickel-transport protein, and the spoT gene, which was recently implicated in bacterial resistance towards U. Detailed genome mining analysis of strain SRS-W-1-2016 also revealed the presence of a plethora of secondary metabolite biosynthetic gene clusters likely facilitating resistance to antibiotics, biocides, and metals. Additionally, several gene homologous for the well-known oxygenase enzyme system were also identified, potentially functioning to generate energy via the breakdown of organic compounds and thus enabling the successful colonization and natural attenuation of contaminants by Arthrobacter sp. SRS-W-1-2016 at the SRS site.
Meta-‘Omic’ Analysis of the Functional Diversity Within A Methane Producing Anaerobic Digester Reveals Sulfate-Reducing Bacteria are Primed to Perform Sulfate Reduction Upon Sulfate Addition

A. R. St. James, R. E. Richardson; Cornell Univ., Ithaca, NY

Abstract Body:

The anaerobic digestion of wastes is globally important in the production of methane as a biofuel. The presence of sulfate in anaerobic digesters (ADs) stimulates the activity of sulfate-reducing bacteria (SRB), decreasing methane production by outcompeting methanogens for common substrates and producing sulfide, a toxic and corrosive byproduct that can negatively impact reactor function. While diverse SRB populations are known to persist in ADs even without sulfate addition, little is known about how the functional diversity of these populations governs varied metabolic responses to sulfate availability. Here we show that SRB populations within a butyrate-fed AD enriched from the Ithaca Wastewater Treatment Plant and maintained for nearly 30 years without added sulfate, immediately respond to sulfate availability and effectively outcompete hydrogenotrophic methanogens, reducing methane production by over 30%. We compare metatranscriptomes from control and sulfate amended subcultures from within the first 48 hours of sulfate availability. We hypothesized that each SRB population would choose one of three responses that can be resolved in the transcriptome: (i) switching from fermentative to respiratory metabolism, (ii) continuing fermentative metabolism, and (iii) continuing respiratory metabolism that had been supported by low-level internal sulfur cycling at sub-micromolar sulfate levels. We link initial transcriptome response to long-term SRB niche partitioning using comparative metagenomic analysis of a sub-enrichment maintained with 2 mM sulfate for 1 year. We demonstrate a significant increase in the representation of enzymes involved in the sulfate reduction pathway (AprAB, dsrABC, QmoABC). Finally, we show that fermentative SRB populations remain at relatively constant levels while a diverse hydrogenotrophic SRB population effectively replaces the obligately hydrogenotrophic methanogen, Methanospirillum hungatei, as the primary hydrogen-utilizing organism. Our results indicate that hydrogenotrophic SRB populations are primed for response to sulfate availability and can effectively outcompete obligately hydrogenotrophic methanogens. These findings elucidate key ecological dynamics between SRB and methanogens which can be applied to a variety of engineered and natural systems, including assisting in the design of more effective ADs, suggesting that inoculation with hydrogenotrophic methanogens may be necessary to speed AD recovery from sulfate stress.
Abstract:
Isolation of A Phenazine Oxidizer from Soil from Woods Hole, Ma
Primary Author Block:
Abstract Body:
Microbial communities cycle electron donors and acceptors, such as oxygen/water, sulfate/sulfide and ferric/ferrous iron, to facilitate their metabolism. Pseudomonads and other soil-dwelling bacteria produce redox-active secondary metabolites, phenazines, which play important roles in signaling, antimicrobial defense, and maintaining intracellular redox homeostasis. However, little is known about the fate or function of phenazines in the environment. Because oxidized phenazine-1-carboxylic acid (PCA) is reduced by diverse soil bacteria, we hypothesized that reduced PCA could serve as an electron donor for other microbes in the soil. We therefore set out to enrich, and later isolate, a soil organism that would oxidize, but not degrade, reduced PCA under anoxic conditions. Our primary enrichment used reduced PCA as the electron donor, nitrate as the sole electron acceptor, and acetate as the non-fermentable carbon source. The inoculum came from soil near the Marine Biological Laboratory in Woods Hole, MA. After streaking an enrichment with the oxidative activity on LB and picking several colonies, we attained a culture of Enterobacteriaceae that completely oxidized 1 mM PCA within 5 days; additional isolation attempts from the enrichment culture are in progress. To determine whether the culture oxidizes PCA as a side-effect or as a necessary part of its metabolism, we tested its growth with and without an electrode poised to re-reduce any PCA the culture oxidized. The preliminary results indicate increased growth in the presence of the electrode. Interestingly, we also found that P. aeruginosa and E. coli have this oxidative activity, at a similar rate to that of the isolate, under the same conditions. This suggests that the oxidation of PCA may be coupled to denitrification, and that the redox cycling of phenazines may be a widely-distributed property of bacterial communities.
Session Number: 433  
Session Type: Poster  
Session Number: 433  
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Session Title: AES10 - Genetic and Metabolic Functions in Environmentally Relevant Microbes: Carving Out A Niche  
Session Start Date Time: 6/10/2018 12:45:00 PM  
Session End Date Time: 6/10/2018 2:45:00 PM  
Session Primary Track: Applied and Environmental Science  
Abstract Control Number: 6529  
Poster Board Number: SUNDAY - 902

Abstract Title:  
Ecofunprimer Tool: Pipeline to Design Primers for Eco-Functional Genes for High-Throughput Qpcr Platforms  
Primary Author Block:  
S. Gunturu, O. Yang, T. D. Carvalho, J. Cole; Michigan State Univ., East Lansing, MI  
Abstract Body:  
Designing primers for ecofunctional genes such as genes involved in nitrogen cycling is challenging due to the diverse alleles present within a given environment. Current methods to target ecofunctional genes often use highly degenerate universal primers sets developed for key enzymes such as nitrogenase. These “universal” degenerate primers often have low efficiency and do not comprehensively cover the highly diverse protein coding genes. Here, we introduce EcoFunPrimer to develop primers for important Ecological Functional genes for high throughput qPCR platforms. For input, EcoFunPrimer will take a list of curated reference sequences for the gene of interest, and set experimental parameters such as primer/amplicon length, melting temperature, and maximum degeneracy per primer pair. Users can quickly filter out regions of the sequence with invalid melting, hairpin, and homodimer temperature with our custom code incorporating the well-known Primer3 tool to develop minimum number of primer pairs to cover the maximum number of references sequences. These sets of primers are ideally suited for use with commercial high-throughput (q)PCR platforms, such as the SmartChip and AccessArray. Users also have the option to provide a phylogenetic tree to calculate sequence weights from a tree to avoid reference bias with overrepresented or underrepresented alleles and to weight references by other biological criteria, e.g. environmental source or prior knowledge of community membership. The software and documentation is available through https://github.com/rdpstaff/EcoFunPrimer
Stimulation by Sublethal Doses of Nanocomposites

Primary Author Block:
A. Augustyniak, K. Cendrowski, M. Barylak, E. Mijowska, P. Nawrotek; West Pomeranian Univ. of Technology, Szczecin, Poland

Abstract Body:
The bacteria-nanomaterial interactions have been frequently studied in terms of toxicology. Nevertheless, the possible impact of sublethal doses of nanomaterials on the metabolic activity of microbial cells is the aspect that still seems to be neglected by most authors [1, 2]. There is some evidence that they can alter secondary metabolism in bacteria, what can have both, medical and biotechnological significance [3]. The main goal of the study was the evaluation of effects caused by sublethal doses of selected nanocomposites on secondary metabolism of environmental and reference bacteria from genera Pseudomonas and Streptomyces. Material for the studies consisted of ten distinctive bacterial strains (five streptomycetes and five pseudomonads). Studied nanomaterials were silica- and carbon-based nanocomposites (including graphene) functionalized with copper, cobalt or titanium dioxide nanoparticles. They were organized in various dimensional structures including nanotubes, nanospheres, or nanoflakes. Bacterial physiology and metabolic activity in response to nanocomposites was tested with the use of spectrophotometry, real-time PCR, phase-contrast and electron microscopy, elemental mapping, and flow cytometry. All methods were supported by a variety of standard microbiological techniques. Nanomaterials concentration was ranging from 0.01 - 0.1 mg/mL. The research was conducted for three years. Nanocomposites present in the growth environment stimulated bacteria to produce secondary metabolites including pyocyanin (Pseudomonas aeruginosa) and medermycin (Streptomyces sp.). One of studied streptomycetes produced some antifungal metabolite when stimulated with graphene oxide with cobalt nanoparticles. Silica nanomaterials altered the ability of pseudomonads to produce biofilm resulting in biofilms expressing higher metabolic activity than controls. Titanium dioxide was found inside Streptomyces cells, whereas in Pseudomonas aeruginosa it increased the expression of the MDR efflux pump. Furthermore, several of studied nanomaterials forced the cells to rapidly agglomerate which could be seen macro- and microscopically. Gathered evidence show that nanocomposites in sublethal doses can stimulate bacterial metabolism leading to shifts in physiology and metabolic activity. This can be problematic in healthcare, because metabolites such as pyocyanin contribute to bacterial virulence. On the other hand, observed phenomena could be used in biotechnological production of desired metabolites.
Bloom of A Denitrifying Methanotroph, "candidatus Methylomirabilis Limnetica", in A Deep Stratified Lake

Primary Author Block:
J. S. Graf1, M. J. Mayr2, H. K. Marchant1, D. Tienken1, P. F. Hach1, A. Brand2, C. J. Schubert2, M. M. M. Kuypers1, J. Milucka1; 1Max Planck Inst. for Marine Microbiol., Bremen, Germany, 2Eawag, Kastanienbaum, Switzerland

Abstract Body:
Microbial oxidation of methane is a key process controlling the flux of methane to the atmosphere in most aquatic systems. In freshwater lakes, methane oxidation is predominantly coupled to oxygen respiration and the indigenous methane-oxidizing microbial community is typically dominated by aerobic methanotrophs affiliated mainly with gamma- and to a lesser extent alpha-proteobacteria. Microorganisms that oxidize methane anaerobically, such as ANME archaea or bacteria of the NC10 clade, often constitute a minor part of the freshwater methanotrophic community and their contribution towards methane oxidation in freshwater lakes is still poorly constrained. We investigated the methane-oxidizing microbial community in a methane-rich, anoxic hypolimnion of a permanently stratified freshwater lake using functional metagenomics, metatranscriptomics and catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH). Our results showed that a novel species of the NC10 clade, “Candidatus Methylomirabilis limnetica”, outnumbered gamma-proteobacterial methanotrophs and dominated the planktonic methanotrophic community in the anoxic depths of Lake Zug in two consecutive years. “Ca. M. limnetica” comprised up to 27% of the total bacterial population, which is the highest abundance so far reported in the literature. Correspondingly, gene transcripts assigned to “Ca. M. limnetica” constituted up to one third of all metatranscriptomic sequences in situ, with functional genes related to denitrification and methane oxidation being among the highest transcribed genes. We reconstructed a high-quality genome of “Ca. M. limnetica” which included a complete pathway for methane oxidation and an incomplete denitrification pathway, including a highly transcribed gene encoding for putative nitric oxide dismutase. We identified features possibly related to genome streamlining (i.e. less redundancy of key metabolic genes) and adaptation to its planktonic habitat (i.e. gas vesicle genes). The genomic and transcriptomic analyses, together with physicochemical parameters and the single-cell metabolic activity measurements provided insight into the metabolic function of this relevant freshwater methanotroph.
Abstract Title: Function of Chemotaxis-Like Signal Transduction Pathway in Azorhizobium Caulinodans

Primary Author Block: Z. Xie, W. Liu; Yantai Inst. of Coastal Zone Res., Chinese Academy of Sci., Yantai, Shandong, China

Abstract Body:

Background: Azorhizobium caulinodans ORS571 has the capability of fixing nitrogen both in the free-living and symbiotic state. In addition, it retains a capacity to induce nodule formation on roots as well as on stems of its host Sesbania rostrata under symbiotic conditions. Analysis of available genome sequence indicates that A. caulinodans relies on a single chemotaxis system in the whole genome. The chemotaxis system (che) contains 5 chemotaxis genes: cheAWY1BR, transcribed in same direction and a putative σ54-dependent promoter was identified upstream of the cheA. The function of the chemotaxis operon (che) in A. caulinodans ORS571 has not yet been established. Methods: The cheA and che cluster deletion mutants (ΔcheA and cheA-R) were constructed, and chemotactic behavioral assays of these mutants were performed. The production of exopolysaccharides and biofilm were measured, and competitive colonization and nodulation were assayed. Results: The CheA or Che signaling pathway controls chemotaxis behavior and flagella-driven motility of A. caulinodans ORS571. In the free-living state, biofilm formation and production of extracellular polysaccharides in ΔcheA and ΔcheA-R mutants were impaired compared with wild type. The ΔcheA and ΔcheA-R mutants were also defective in competitive adsorption and colonization of the root surface of host plants. In addition, a functional CheA or Che promoted competitive nodulation on roots and on stems. Interestingly, a non-motile mutant (ΔfliM) was also impaired in biofilm formation, extracellular polysaccharides production and competitive colonization and nodulation. These findings suggest that through controlling flagella-driven motility behavior, the chemotaxis signaling pathway in A. caulinodans coordinates biofilm formation, extracellular polysaccharides, and competitive colonization and nodulation ability were tested. Conclusions: CheA or Che pathway regulates flagella-mediated chemotaxis behavior, extracellular polysaccharides and biofilm formation in free-living state, and these behaviors directly influence the competitive colonization and nodulation of the host plant.
Abstract Title:
Exploring the Impacts of Environmental Microbiota on Microbial Succession and Metabolic Profiles During Chinese Liquor Fermentation

Primary Author Block:
H. Du, X. Wang, Y. Xu; Jiangnan Univ., Wuxi, China

Abstract Body:
Background: In the modern age, most traditional craft production of fermented foods and beverages has been replaced by partial or full industrialization. Spontaneous food fermentation can hardly be controlled without understanding the effect of environment on microbial succession and metabolism. Chinese liquor fermentation is a spontaneous solid-state fermentation process driven by natural microbiota. The type of process used to make liquor—craft or industrial—alters the operational environment and the aromatic qualities of the product contributed by various microbial consortia, but differences in microbial community assembly and temporal succession are often overlooked.

Methods: In this study, we investigated bacterial community dynamics, substrate consumption, and metabolite production during both craft liquor-making process (CLP) and industrial liquor-making process (ILP). The V3-V4 region of the 16S rRNA gene was amplified using universal primers. The PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter, CA, USA), and then subjected to 300-bp paired-end sequencing on a MiSeq Benchtop sequencer (Illumina, San Diego, CA, USA).

Results: The change of environmental microbiota could drive both microbial succession and metabolic profiles during liquor fermentation. The compositions of bacterial communities were different in bacterial species between CLP and ILP at the beginning of fermentation. During ILP, glucose was used more rapidly by microbiota, leading in turn to a higher ethanol production rate during the early stage of pit fermentation. The higher rate of ethanol production in ILP shortened the lifetime of bacteria such as Weissella, Pediococcus, Leuconostoc, and Bacillus during the early stage of fermentation. Lactobacillus sp. became dominant earlier in ILP than in CLP. Finally, the change in bacterial community dynamics led to changes in aroma compounds.

Conclusion: We found that environment was the source of many important microorganisms for Chinese liquor fermentation. The findings provide feasibility to better control spontaneous food fermentation.
Abstract Title:
Characterization of Microbial Communities in Soil and Water Column of Treatment Wetlands: the Everglades Storm Water Treatment Area 2a As A Case Example

Primary Author Block:

Abstract Body:
Eutrophication of fresh water through human activities is a global problem. In South Florida, large agricultural areas needed to sustain growing populations are upstream of the Everglades and create the potential for runoff of excess fertilizers. In response, the Everglades Storm Water Treatment Areas were developed to reduce nutrient load before water flows into the Everglades. These constructed wetlands use a variety of mitigation strategies, including different vegetation types. Here, we examine the microbial community composition through 16S illumina sequencing of two different types of constructed wetlands to better understand how microbial nutrient cycling impacts nutrient removal from the water column. We sampled soil cores and overlying water column from a constructed wetland with two cells of different vegetation types: submerged aquatic vegetation (SAV) and emergent aquatic vegetation (EAV). A total of 79 microbial phyla were detected, including 21 phyla that were detected at greater than 1% relative abundance. Vegetation type showed a significant effect not only on alpha diversity but also on microbial composition, especially in the water column. Alpha diversity is higher in EAV compare to SAV. Within a cell, alpha diversity is highest in upper soil levels and lower in the water column and deeper/older soil that was present before the construction of the wetland. Microbial compositions in both cells are clustered by soil depth and N:P ratio and vegetation types. Water samples of the two cells were distinct but more similar to each other than to soils. Characterization of the microbial communities within soil profiles and across the flow-ways of constructed wetlands provides a baseline upon which we can test the impact of nutrient runoff mitigation.
Abstract Title:
The Responses of Microbial Communities to Climate Warming: Results from Long Term Soil Incubation

Primary Author Block:
L. Y. Wu1, H. Yin2, R. Bracho3, E. A. G. Schuur4, Y. Luo4, X. Guo1, W. Shi1, J. Zhou1; 1Univ. of Oklahoma, Norman, OK, 2Central South Univ., Changsha, China, 3Univ. of Florida, Gainesville, FL, 4Northern Arizona Univ., Flagstaff, AZ

Abstract Body:
Background: The world's soils contain twice as much carbon as the atmosphere. As a result, small increases in organic carbon loss from soils could greatly enhance carbon dioxide concentrations in the atmosphere. Changes in the microbial temperature sensitivity of decomposition of carbon will have a significant effect on the soil carbon balance. Methods: Long-term incubations were performed (15 & 25°C) to determine the temperature sensitivity of microbial respiration and the microbial phylogeny and functional dynamics in soils from a temperate grassland site (Oklahoma, OK; warmed with or without roots, and controls). Carbon fluxes were measured periodically over the course of the incubation, and Q10 was estimated. The microbial community phylogenetic and functional composition, structure, and dynamics were analyzed by 16S rDNA sequencing and functional gene array GeoChip 5.0. Results: Results showed that after one year of incubation, total C respired accounted for 5% at 15°C and 10% at 25°C of the initial soil C content after one year of incubation. Fast decomposing C reached up to 4% of the initial soil C content at 25°C. The overall Q10 was 3.2 ± 0.3. Significant warming effect on microbial communities was observed based on 16S rDNA sequencing. After two weeks at 250°C, samples from the warmed soil with root excluded had more abundant populations of Acidobacteria, Actinobacteria, Crenarchaeota, Firmicutes, Gemmatimonadetes, Nitrospira, Planctomycetes, Verrucomicrobia, but Armadimonadetes, Bacteroidetes, Chloroflexi, Proteobacteria were less abundant; however after nine months incubation, the community differences disappeared. At the bacterial phylum level, no significant warming effect was observed for the soils with roots. GeoChip results showed significant differences in microbial functional diversity. Conclusions: These results indicate that the warming effects were more significant when roots were excluded and for those populations responding to labile carbon.
Abstract Title: Brown Algae Polysaccharide Assimilation Potential in Intertidal Sediments of A Subantarctic Sheltered Environment

Primary Author Block: H. M. Dionisi, J. A. González, P. A. Calderoli, M. Lozada; Ctr. for the Study of Marine Systems (CESIMAR), Puerto Madryn, Chubut, Argentina

Abstract Body:
Brown algae dominate high-latitude coastal environments. As polysaccharides constitute up to 50% of their dry weight, these biopolymers represent an important carbon source for sediment microbial communities. Alginates (1,4-linked β-D-mannuronate and α-L-guluronate) and fucoidans (fucose-containing sulfated polymers) are the two main matrix polysaccharides of their cell wall. We investigated the potential of microbial communities from intertidal sediments of Ushuaia Bay (Tierra del Fuego Island, Argentina) to assimilate these polysaccharides, in order to increase our understanding of the microbial populations that drive this important metabolic process of the carbon cycle, and the enzymatic mechanisms that they use. Sequences potentially coding for alginate- and fucoidan-depolymerizing enzymes were mined in a sequenced metagenomic library (0.7 Gb, N50 25 Kb), using dbCAN, pfam and custom-built HMMs. Putative alginate lyase and oligoalginate lyase sequences were very abundant in the dataset (1 every ~2,700 sequences). Sequences belonged to 6 of the 7 polysaccharide lyase (PL) CAZy families that include alginate depolymerizing enzymes. The scaffolds containing these sequences were assigned to 10 different phyla (PhylopythiaS), with Bacteroidetes (28.6%), Proteobacteria (24.9%) and Planctomycetes (17.4%) being the most abundant. Sequences of PL5 and PL14 families were most often found in scaffolds assigned to Planctomycetes, while those from the PL6, PL7, PL15 and PL17 families were most often identified in scaffolds assigned to Bacteroidetes. In agreement, correspondence analysis showed an association between certain PL families and the corresponding phyla, suggesting a differential contribution of guild members to the alginate depolymerizing gene pool. Putative fucoidanase sequences were less abundant (1 every ~97,600 sequences), although probably underestimated due to the lack of diversity of available sequences for HMM construction (GH107, 8 sequences from 4 bacterial strains). The identified metagenomic sequences shared low to medium identity values with members of the GH107 family (9-40% at protein level), and were often located in clusters that included putative alpha-L-fucosidase, sulfatase and acetyl esterase sequences, in particular those assigned to the Planctomycetes phylum (57% of the scaffolds). These results provide a community-wide profile of the potential for the degradation of key components of brown algae biomass, and constitute the foundation for their biotechnological application.
Abstract Title:
Efficiency of Uv Sunscreens from Cyanobacteria
Primary Author Block:
S. Nosheen, M. Ahmed; Univ. of the Punjab, Lahore, Pakistan
Abstract Body:
Background: Ultra violet (UV) radiations are essential component of Sun's electromagnetic spectrum that is harmful for living organisms. Fortunately most of these hazardous radiations are filtered before reaching the earth surface. Ozone layer is continuously decreasing due to increasing atmospheric pollutants that result in increased UV radiations reaching on the earth surface. Living organisms have different mechanisms to cope up with hazardous UV radiations. One of the mechanisms is production of UV protective compounds. In Cyanobacteria, being one of most primitive but successful living organism, production of UV protective compounds is a very competent defense mechanism. Current study deals with the analysis of UV protective compounds from cyanobacterial isolates. Methods: UV protective compounds were extracted from 20 isolates and their UV absorption spectrum was analyzed. Five strains producing significantly higher quantity of compounds were selected and their ability was analyzed under UV and nutrient stress. MAA's and scytonemin were characterized by GC/MS. UV protective ability of compounds was checked against three test bacteria (E. coli, S. aureus and Proteus mirabilis). DNA damage protection ability was checked by Comet assay. Results: Isolates produced varying quantity of photo-protective compounds. Various peaks of absorbance were observed, in all three UV regions (A, B and C). The observed trend according to number of peaks was UV-C (less than 280nm) > UV-B (280-315 nm) > UV-A (315-400 nm). UV and nitrate stress significantly induces the production of UV protective compounds. UV exposure not only increased the number of peaks but also the absorbance values with a clear-cut trend shifts toward the production of more UV-B and UV-A compounds as compare to control. Supplementation of the isolated UV protective compounds significantly increased (up to 2 folds) the viability of E. coli, S. aureus and Proteus after lethal UV exposure. Comet assay showed significant decrease in UV induced DNA damage in presences of these compounds. Conclusions: Cyanobacteria from unexplored and stressed environment can be source of UV protective compounds that can have multiple applications in various fields.
Staphylococcus aureus Prompts Escherichia coli Survival Under Dry Conditions: A Potential Threat from the Viewpoint of Nosocomial Infections

Primary Author Block:
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Abstract Body:
Background: High-touch places are a threat referred to “hot spots” facilitating the spread of hand-attached bacteria in hospitals, responsible for nosocomial infection. Therefore, both ATP and stamp methods have developed to evaluate the high-touch places with bacterial contamination quickly. Meanwhile, our previous studies showed unintelligible a prominent gap between the amounts of ATP and bacterial colony in hospital floors maintaining dry conditions, presumably underlying unfitted method or unknown bacterial dynamics under dry. Hence, we evaluated bacterial dynamics on dry-floor materials at distinct temperatures by the ATP and stamp methods with accurate monitoring bacterial numbers. Methods: Staphylococcus aureus ATCC 29213 and Escherichia coli ATCC 25922 were separately suspended, and mixed together (or not) in LB broth. The 20μl of each solution (5.0 × 10⁵ CFU/spot) were then spotted on the flooring materials (3cm × 3cm). They were then dried and incubated at distinct temperatures (room, 30°C, 37°C) up to 11 days. After incubation, dried spots were wiped with cotton swabs, and suspended into PBS. The amounts of both bacteria was evaluated by spreading out on Mannitol salt agar and MacConkey agar, expressed as CFU per spot. Furthermore, the spots were visualized by the observation with SEM. The amounts of ATP on each spot was also monitored by Clean-Trace Luminometer (3M, USA). Results: As expected, in the dry case of single suspension, S. aureus endured with a gradual decrease of CFU over time, although E. coli rapidly died until 4 days. Meanwhile, in the dry case mixed with S. aureus, E. coli survived more longer time; it tended to come stronger under low temperature. However, such bacterial dynamics could not be detected by the ATP measure, regardless of single or mixed. SEM revealed no morphological differences of both bacteria between single and mixed cases. Conclusions: We concluded that S. aureus prompts E. coli survival under dry conditions, responsible for a potential threat from the viewpoint of nosocomial infection. Also, ATP measure likely discouraged us from accurately implementing hospital cleanness against nosocomial infection.
Spatial and Longitudinal Influences on Accurately Predicting A Microbiome Biofingerprint

Primary Author Block:
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Abstract Body:
Background: Microorganisms in the indoor environment can be attributed to complex interactions between outdoor conditions, indoor conditions, and occupants. The occupant contribution has been found to be the most significant and therefore we hypothesis a better understanding of the occupant microbiome and the built environment is needed for human health, future sampling methodologies, and predictive applications. Methods: Specifically, this study conducted a longitudinal sampling effort across six weeks with 22 participants. For each participant, their hand and various office and home surfaces were sampled, including the hand of their significant other. Bacterial DNA was retrieved from 264 samples and 16S DNA was amplified, sequenced using bi-directional Illumina MiSeq, and analyzed in QIIME. Models to include Random Forest, Source Tracking and others predicted which human hand bacterial microbiome was predominately in contact with each surface. Results: The hand and office environments were dominated by known human skin genera including Streptococcus, Staphylococcus, Corynebacterium, and Pseudomonas. Across the weeks, the microbial community did alter but not in a predictable manner. The human skin signature was also present in the home environment, more detectable on the nightstand compared to vacuum samples of the living and bedroom areas. The computer mouse and keyboard had the same community of bacteria, despite the additional contact time with the mouse and the skin. The office and home samples were four- to ten-fold more predictive than by chance of the occupant when analyzed through supervised learning. Interesting, the computer mouse microbiome sample was the best predictor of the occupant, even better than the occupant’s own hand, possibly related to community stability that should be considered in future biofingerprinting studies. Conclusions: The results from this pilot study suggest approaches for predicting occupancy and also give a broader understanding of how microbes are passed between occupants and the built environment.
Abstract Title:
Microbial Monitoring in A Pharmaceutical Manufacturing Facility: A Space Habitat Analogue

Primary Author Block:
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Abstract Body:
Astronauts already stay in the International Space Station (ISS) for several months to a year, and manned Moon and Mars missions may be realized within the next two decades. To realize long-term space habitation, accumulation of fundamental knowledge on microorganisms in the environment is essential to ensure safety and healthy lives; accordingly, research has been carried out in the ISS. To estimate the potential risks of microbes existing in the space habitats, and because the opportunities for space experiments are limited in the ISS, we need to repeat experiments in a model environment on Earth. As a controlled and confined environment, pharmaceutical manufacturing facilities are considered as an effective model environment for space habitation, aside from differences in gravity and radiation. Microbes in such facilities are controlled under GMP regulations, and the ecosystem is mainly composed of humans and microbes. Therefore, in this research, we aimed to determine the bacterial abundance in a pharmaceutical manufacturing facility as a model of a space habitat using conventional culture methods, a microbial particle counter, and quantitative PCR (qPCR). The microbial particle counter used in this study measures physiologically active microbial particles based on their autofluorescence signals. Furthermore, to understand the environmental microbiome in the facility, the bacterial and fungal community structures were determined by high-throughput rRNA gene targeted amplicon sequencing. The microbial particle number determined by a microbial particle counter was equivalent to that determined by qPCR on samples from 80 m3 of air obtained by a high-volume air sampler. The ratio of microbial count to particles was approximately 5% on average, increased according to cleanliness. Human related-microbes, such as Actinobacteria, Firmicutes, Gammaproteobacteria and Malassezia dominated, as they do in the ISS. From the above, we suggest that risk management of human-related microorganisms is important in controlled and confined environments such as pharmaceutical manufacturing facilities and space habitats. These results provide fundamental data for the evaluation and control of microbial quality in space habitats, as well as for the pharmaceutical and food industries.
Abstract Title:
Isolation and Characterization of A Algicidal Bacterium against the Harmful Algae Bloom (Hab) Species Chattonella Marina

Primary Author Block:
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Abstract Body:
Harmful algal blooms (HABs) have made massive economic losses and marine environmental disturbances in Korea. Normally, loess was scattered to control HABs in Korea, but it arises secondary contamination problems. Although these are several research articles and patents are dealing about algicidal bacteria and chemicals in Korea, related with cause of its efficiency and safety. However, it has not been applied on practical site before. Therefore, development of highly effective strategies are required to control the HABs. In the present study, we isolated 101 bacterial strains from sea water and sediment in Tongyeong and Pohang, Korea. From these stains, we tested algicidal test against Chattonella marina. As a result, strain TF15 was selected for high algicidal activity against Chattonella marina. This bacteria belonged to Roseivirga genus. Supernatant of Roseivirga TF15 showed >79% algicidal activity within 6h, >95% algicidal activity within 24h. The TF15 had highest algicidal efficiency (>97.5%) when the cell density of TF15 was 0.9 in 600nm absorbance. Algicidal activity of Roseivirga TF15 was analyzed against to 6 kinds of major algae causing harmful algal bloom (HAB) in Korea such as Akashiwo sanguinea, Heterosigma akashiwo, Prorocentrum minimum, Heterocapsa triquetra, Cochlodinium polykrikoides, Skeletonema costatum. From the result, Roseivirga TF15 showed selective algicidal activity against Chattonella marina, Akashiwo sanguinea, Heterocapsa triquetra and it had no efficiency about other 4 kinds of harmful algae. Summarily, this study proposed that Roseivirga TF15 has potential algicidal bacteria to control HABs in ocean.
Abstract Title:
The Role of Small Intestinal Bacterial Overgrowth in Pathogenesis of Lactase Deficiency

Primary Author Block:
N. Fadeeva, I. Ruchkina, A. Parfenov, P. Shcherbakov, O. Knyazev; Moscow Clinical Res. Ctr., Moscow, Russian Federation

Abstract Body:
Background: Secondary lactase deficiency (SLD) is inability to digest lactose. This inability results from decrease of lactase enzyme activity, which is produced in the small intestine. Aim: to define the influence of small intestinal bacterial overgrowth (SIBO) in adult patients with SLD. Methods: In this study, 386 patients (the mean age - 33.9±9.09; F/M 249/137) with postinfectious irritable bowel syndrome (IBS) were analyzed concerning lactase deficiency. All patients underwent intestinal endoscopy with biopsies from the mucosa of the descending duodenum to determine lactase deficiency. The biopsies were taken to determine lactase deficiency (normal, mild, and severe) by means of lactose quick test (LQT). To diagnose SIBO all patients underwent lactulose breath test for 2 hours. Results: SLD was detected in 36.5% of patients with postinfectious IBS. SLD in all cases was accompanied by SIBO. It turned out that the degree of lactase deficiency depends on the severity of SIBO in the lumen of the small intestine. Thus, when mild SLD average value SIBO was 72.4±25.1 ppm, whereas severe SLD average indicators of SIBO achieved higher values, 99.3±26.9ppm (N≤20ppm). The inverse correlation between the degree of lactase deficiency in patients with the SLD and the severity of SIBO in the small intestine was detected, i.e. the higher the hydrogen concentration in the exhaled air, the less activity of the enzyme lactase in the small intestine biopsy specimens (r= -0.49, p<0.001). Sixty patients with mild SLD were divided into two groups. The first group (41 patients) was taken probiotic (composed of Bifidobacterium longum 1*10^7, Enterococcus faecium 1*10^7) for 2 weeks. The second group (19 patients) was taken placebo for 2 weeks. The study was randomized double-blind controlled in parallel groups. In group patients who took the probiotic during 14 days (41 patients) in 29 patients (70.7 %) were registered decrease of the clinical symptoms (p<0.0001); decrease of the lactulose breath test level (p<0.01) and negative LQT (p<0.01). In 29,3% patients weren’t registered any changes. In group patients who took placebo for 14 days there was no positive effect in 68.4% of cases. Conclusions: The high frequency of the SLD associated with SIBO in the small intestine in patients postinfectious IBS can be explained by the growth of pathogenic microflora in the small intestine. The probiotic composed of Bifidobacterium longum 1*10^7, Enterococcus faecium 1*10^7, demonstrated efficiency in correction of SLD and can be used to prevent SIBO.
Abstract Title:
Glycocalyx slime-producing and autotrophic bacteria used in self-healing technology for concrete cracking

Primary Author Block:
Y. Park, H-S. Kim, J. Kim, S. Kang, S. Jeong, S-S. Lee; Kyonggi Univ., Suwon, Korea, Republic of

Abstract Body:
Development of self-healing concrete is required to recover concrete-cracking because the technique can solve the problems in economic aspects. This study is to develop a self-healing technique for concrete-cracking during the long term by using, which can be tolerance to strong alkali environment. Glycocalyx slime-producing bacteria were selected in the previous study. Rhodobacter capsulatus (KACC 15298), Rhodobacter blasticus (ATCC 33485) were used to distinguish slime due to the carbon source change. Both strains with 50ml of each strain and 5L of each culture with two kinds of carbon source (succinate, malate). Strains incubate at 28±2°C for 30 days under light. Slime components from each bacteria strain were analyzed by high-performance liquid chromatography (HPLC), gas chromatography-flame ionization detector (GC-FID), enzyme-linked immunosorbent assay (ELISA). When strains used succinate for carbon source, R. capsulatus had 10.0 ± 0.006 mole% of neutral sugar, 1.0 ± 0.009 mole% of uronic acid and 22.2 ± 0.003 mole% of protein. R. blasticus had 9.3 ± 0.002 mole% of neutral sugar, 1.6 ± 0.002 mole% of uronic acid and 21.9 ± 0.006 mole% of protein. When strains used malate for carbon source, R. capsulatus had 8.0 ± 0.007 mole% of neutral sugar, 1.8 ± 0.006 of uronic acid and 12.5 ± 0.009 of protein. R. blasticus had 5.4 ± 0.002 mole% of neutral sugar, 1.2 ± 0.002 mole% of uronic acid and 11.3 ± 0.005 mole% of protein. Thus, both strains showed more protein and neutral sugars in slime produced using malate as a carbon source than succinate. For analysis of slime molecular weight, when each bacteria strain incubated with using succinate as a carbon source, R. capsulatus was measured as 348.3 kDa and R. blasticus as 687.9 kDa. When using malate as a carbon source, both strains had not effective molecular weight. Therefore, R. capsulatus was composed of small slime molecular weight at the same amount, molar concentration was found to form more slime than R. blasticus. Evaluation of the amount of slime production, when succinate was used as a carbon source, R. capsulatus produced 2.57 g/L of slime, R. blasticus produce 1.03 g/L of slime. When malate was used as a carbon source, R. capsulatus produced 9.54 g/L of slime, R. blasticus produced 3.74 g/L of slime. Therefore, R. capsulatus using malate as a carbon source showed the highest slime product efficiency.
Abstract Title:
Retrieving Microbial Dna from Human Touched Objects for the Microbiome-Based Forensic Applications

Primary Author Block:

Abstract Body:
Trace evidence analysis includes the identification and comparison of transferred materials, which is a core of forensic science and has played a crucial role in forensic investigations. Recent applications of the microbiome as trace evidence in forensic sciences raised a question on which human touched object is more suitable for the microbiome-based analysis. To address this question, we isolated DNA from trace evidence and measured the quantity and quality of DNA from objects commonly found in a workplace environment. We chose to use several objects, such as cabinet handle, cell phone, computer monitor, doorknob, keyboard, mouse, office phone, and stapler and extracted DNA from those objects using three different commercial kits. DNA was then quantified to identify which human touched objects is more suitable for microbiome-based applications and which DNA extraction kit yields large quantity and high quality DNA from the objects. Based on the DNA quantity measured by a spectrophotometer, there was no significant difference observed between the kits based on two tailed t-test. Although DNA yields were low, mouse showed the highest DNA yield followed by doorknob, cell phone, keyboard, and office phone. The 16S rRNA gene sequencing is necessary to identify the diversity of the microbiome associated with each object.
Abstract Title:
Use of Tuned Polymers for the Enhanced Retention of Bacteria in Cleaning Wipes

Primary Author Block:
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Abstract Body:
Cleaning and disinfection are the first line of defense used by environmental services personnel for control of the transmission of pathogens. The means by which surfaces are cleaned can have a profound effect on the outcome of these interventions. Wiping, either with a towel, mop, or sponge are common ways to clean a surface. The wiping action provides the force required to remove contaminates from the surface, but can also spread or smear contaminates across the surface, increasing the potential for transmission of pathogens. Therefore, it would be advantageous if a cleaning wipe could not only remove contaminates from a surface but retain those contaminates, reducing the likely hood of inadvertently spreading bacteria. A total of 53 polymers were synthesized with various backbones comprising of polymethylacrylate (PMMA), polysiloxane (PSiOH), polymethylsiloxane (PMSiO) or polyacrylate (PMA). To these backbones phenylalanine and lysine amino acid side chains were incorporated via a carbon linker (0 to 6 carbons in length) to provide a tunable cationic and lipophilic character. Synthesized polymers were screened for solubility and their effect on the viability of Escherichia coli ATCC11775 and Salmonella typhimurium ATCC 14028 in suspensions. Polymers were screened for their trapping effect against E. coli and Salmonella incorporated into either a cellulose substrate or a cleaning wipe. In addition, their ability to bind to LPS was measured as well. 14 polymers were able to effectively bind LPS of both E. coli and S. typhimurium. 4 water soluble polymers were able to bind to E. coli when treated on a cellulose substrate. 3 polymers were able to bind to E. coli and 5 polymers were able to bind S. typhimurium when treated on paper towels. The best performing polymers had a PMMA backbone with a 3:1 lysine to phenylalanine ratio with a 0 and 2 carbon linker for lysine and phenylalanine side chains, respectively. 1 polymer had a free carboxy acid while the other had a methylester moiety. The polymers were able to trap 92.2% ± 1.6% and 99.8%± 0.1% (n=3), E. coli and S. typhimurium, respectively. LPS binding was best accomplished with PMMA or PMA backbones with a ratio of 2:1 or lower of lysine to phenylalanine with no linker. It is possible to achieve a > 95.8% retention of E. coli and S. typhimurium with a polymer tuned to have an optimal cationic and lipophilic balance. This balance also helps with retention of the polymer to the cellulose wipe materials. Ultimately, providing a new cleaning tool to reduce the potential of infectious disease.
Abstract Title:
Evidence for the Co-Occurrence of Nitrite-Dependent Anaerobic Ammonium and Methane Oxidation Processes in the Wastewater Sludge of China: Community Structure and Seasonal Dynamics

Primary Author Block:
S. Xu1, W. Lu2; 1Key Lab. for Solid Waste Management and Environment Safety (Tsinghua Univ.), Ministry of Ed. of China, Beijing, China, 2Sch. of Environment, Tsinghua Univ., Beijing, China

Abstract Body:
Anaerobic ammonium oxidation (ANAMMOX) and denitrifying anaerobic methane oxidation (DAMO) have been recently discovered as relevant processes in the carbon and nitrogen cycles of wastewater treatment plants. In this study, the seasonal dynamics of ANAMMOX and DAMO bacterial community structures and their abundance in sewage sludge collected from wastewater treatment plants were analysed using the specific primers for ANAMMOX and DAMO bacteria. Results indicated that ANAMMOX and DAMO bacteria co-existed in sewage sludge in different seasons and their abundance was positively correlated (P<0.05). The high abundance of ANAMMOX and DAMO bacteria in autumn and winter indicated that these seasons were the preferred time to favour the growth of ANAMMOX and DAMO bacteria. The community structure of ANAMMOX and DAMO bacteria could also shift with seasonal changes. The “Candidatus Brocadi” genus of ANAMMOX bacteria was mainly recovered in spring and summer and an unknown cluster was primarily detected in autumn and winter. Similar patterns of seasonal variation in the community structure of DAMO bacteria were also observed. Group B was the dominant in spring and summer, whereas in autumn and winter, group A and group B presented almost the same proportion. The redundancy analysis revealed that pH and nitrate were the most significant factors affecting community structures of these two groups (P<0.01). This study reported the diversity of ANAMMOX and DAMO in wastewater treatment plants that may be the basis for new nitrogen removal technologies.
Abstract Title:
Quality Assessment of Bottled Water Locally Available in Kathmandu Valley
Primary Author Block:
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Abstract Body:
Background: In recent years, bottled water have been considered as a safer alternative to tap water. This study was carried out to assess the physicochemical and microbiological parameters of bottled water in Kathmandu valley. Methods: A total of 100 samples (50 samples from 20L bottled water and 50 samples from 1L bottled water, in both monsoon and post-monsoon season) were purchased from the local market (bottled water of 20L and 1L were from the same company). The physicochemical and microbial assessment was done as per the methods described in American Public Health Association, 2005. Antibiotic susceptibility test was done for the isolated bacteria. Results: Except for pH and turbidity all the physicochemical parameters of bottled water were found to be within Department of Food Technology and Quality Control (DFTQC) guideline and International Bottled Water Association (IBWA) guideline respectively. The microbial assessment revealed that out of 100 samples, 24(24%) bottled water of 20L and 2(2%) bottled water of 1L were found to be unacceptable according to heterotrophic plate count and total coliform count, in both monsoon and post-monsoon season respectively as per DFTQC and IBWA guidelines. Out of those 52(52%) unacceptable bottled water of both 20L and 1L, 24(24%) and 10(10%) bottled water of 20L contained fecal coliform(s) in both monsoon and post-monsoon season respectively. Out of those 52(52%) unacceptable bottled water of both 20L and 1L, 16(16%) and 7(7%) bottled water of 20L were found to be contaminated with Pseudomonas aeruginosa, in both monsoon and post-monsoon season respectively. However, coliphage, parasite(s), Salmonella species, Vibrio species were absent in all 100 samples. After performing antibiotic susceptibility test, multi-drug resistant Escherichia coli, Enterobacter aerogenes, and P. aeruginosa were screened. Conclusions: Based on the results of assessment; pH, turbidity, heterotrophic plate count and presence of coliforms were the cause of unacceptability of bottled water (both 20L and 1L) as per DFTQC and IBWA guidelines. Fecal coliform(s) and Pseudomonas species was also found in 20L bottled water and were absent in 1L bottled water even though both the bottled water were of the same company and manufactured in the same season. The chemicals and trace elements detected in water have a specific role in our body like electrolyte balance, enzyme and hormone formation, formation and mobilization of vitamin etc.
Prevalence of Antibiotic Resistance Genes (Args) among Children Under 5 in Informal Urban Maputo, Mozambique

Primary Author Block:
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Abstract Body:
Antimicrobial resistance (AMR) is a growing concern in low-income, urban slums where pathogen risks are high and antibiotic stewardship is poor. The role of infrastructure and environmental conditions on the transmission of AMR is not well understood. We examined key AMR gene (ARG) prevalence and concentration in stool samples collected in an existing pediatric cohort in Maputo, Mozambique, part of the MapSan sanitation health impact trial. ARGs of interest include those associated with resistance to beta-lactams (BLA-TEM), chloramphenicol (FloR), quinolone (QnrB), sulfonamide (Sul1), and tetracycline (TetA) antibiotics, as well as a mobile genetic element (IntI1). Droplet digital PCR data so far (147 of 400 total samples) indicate 98% of samples are positive for TetA and 64% are positive for QnrB, demonstrating high prevalence of resistance to these classes of antibiotics in gut flora. Across children, we observed up to 5 log10 variability in gene copies across samples. No significant association was found with the age of the child; further risk factor analysis is ongoing. Surveys of environmental conditions and behaviors will allow for examination of differences before and after a sanitation intervention. We will also assess correlations between enteric pathogen detection and ARG concentrations in stool. Overall, ARGs were very prevalent across ages, and further analyses of ARGs may enhance understanding of the role of environmental exposures at an early age on AMR in children's gut bacteria.
Session Title: AES12 - Microbiology of the Built Environment
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/10/2018 2:45:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 6882
Poster Board Number: SUNDAY - 923

Abstract Title:
Flooding is Associated with Increase in Relative Abundance of Aspergillus and Penicillium Spp. in Built Environment: Implications to Human Health

Primary Author Block:
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Abstract Body:
United States experiences approximately 5 million cases of respiratory problems that are largely correlated with exposures to Aspergilli and Penicillium in built environments. This leads to an estimated healthcare cost of approximately $3.5 billion. Since Americans spend 90% or more of their time indoors, mold related health costs are likely to increase with the rise in weather events such as hurricanes and tidal floods. Current evidence suggests that Aspergillus and Penicillium are largely associated with water-damaged buildings. However, it is not understood whether flooding caused by such extreme weather activities determine the relative abundance of these mold genera in built environment. To address this knowledge gap we conducted a survey of flooded and non-flooded buildings (both residential and non-residential; n=6 per group) that were impacted by two major weather events in South Carolina: the ‘100 year flood’ and hurricane Matthew. Air sampling in these moldy buildings were performed using ‘settle plate’ technique in which the number of mold colonies growing on potato dextrose agar plates upon 30 min of air exposure were analyzed. Colonies that grew on the plate after 5-7 days of incubation at 30°C were identified colony-by-colony using internal spacer sequence 1 (ITS1) analysis coupled with phylogenetic identification of the species. Our results demonstrated significant increase (> 2 fold) in relative abundance of Penicillia and/or Aspergilli in homes where mold problems were flood-associated, as compared to non-flooded homes. Finally, we investigated indoor air samples of buildings (n=6) in New Jersey, which experienced mold problems five years after flooding post hurricane Sandy. We observed a 90% abundance of Penicillium spp. in all these homes, suggesting that the dominance of flood-associated rise of these genera in built environment is long-lasting. Finally, a retrospective review of existing literature on the most abundant Aspergillus/Penicillium spp. identified in our survey include A. flavus, A. fumigatus and P. rubens which are well known pathogens and associated with fatal respiratory and neurological illnesses in immunocomprised patients, children and the elderly. Our findings support our proposed model that contaminants in floodwater may provide new nutrient source for selective overgrowth of pathogenic molds from genera Aspergillus and Penicillium, their sporulation and the emission of their VOCs, which can collectively deteriorate the indoor air quality and detrimentally impact human health.
Abstract Title:
Microbial Enzymatic Activities: Indices of Aesthetic Discoloration on Painted Walls
Primary Author Block:
O. F. Obidi, M. N. Igwo-Ezikpe, F. O. Okekunjo; Univ. of Lagos, Nigeria, Lagos, Nigeria
Abstract Body:
Background: In recent times, reports have shown that microbial enzymes are involved in biodegradation of many substrates. A total of eight pigmented microorganisms were isolated from biodeteriorating painted walls and identified with the 16S rDNA analysis in a previous study. Methods: In this study, we focused on determining the production of phosphatase, endoglucanase and exoglucanase by the isolated strains using standard methods. We also investigated the various conditions for optimal enzymatic activities of the isolated strains in terms of pH and incubation temperature, as well as substrate concentration and incubation time to elucidate their involvement in discoloration of painted walls. All the strains which included the genera Aspergillus, Meyerozyma, Candida, Fusarium, Cerrena and Pseudomonas produced phosphatase, exoglucanase and endoglucanase at different pH, temperature, incubation time and substrate concentration which were used as biomarkers for microbial metabolic activity. Results: The order of optimal production at varying conditions was observed to be: Phosphatase; Cerrena sp > Aspergillus sp > P. aeruginosa > M. guilliermondii > A. aculeatus > F. proliferatum > C. tropicalis > M. caribbica; Exoglucanase: P. aeruginosa > M. caribbica > C. tropicalis > Aspergillus sp > Cerrena sp > M. guilliermondii > A. aculeatus > F. proliferatum; Endoglucanase: Cerrena sp > F. proliferatum > P. aeruginosa > M. caribbica > A. aculeatus > M. guilliermondii > Aspergillus sp > C. tropicalis. Conclusions: Results of microbial enzymatic activity confirm the hypothesis that heavy pigmentation and enzyme production are major indices of discoloration on biodeteriorating painted walls.
Abstract Title:
Water Quality and Microbial Dynamics in A Large Building Hot Water Sys. and Managing Potential Risk Associated with Legionella

Primary Author Block:
V. Gomez-Alvarez1, M. Berberich2, L. Boczek1, D. King1, A. Pemberton1, S. Pfaller1, M. Rodgers1, J. SantoDomingo1, R. P. Revetta1; 1U.S. Environmental Protection Agency, Cincinnati, OH, 2Pegasus Technical Services, Inc. c/o US EPA, Cincinnati, OH

Abstract Body:
Premise plumbing (PP) includes that portion of the drinking water distribution system (DWDS) connected via the service line to houses and other buildings. Water quality in PP is not monitored by U.S. EPA regulation with the exception of the Lead and Copper Rule. Because public health data shows that a significant fraction of the nation’s waterborne disease outbreaks are attributable to PP systems, it is important to understand the characteristics of these systems which amplify the potential public health risk relative to the DWDS. The present study was conducted in a 40-year-old large building supplied with treated chlorinated water with a main and secondary hot water network. We used physico-chemical parameters, heterotrophic plate counts (HPC), and 16S rRNA sequencing data to generate water quality profiles of the bulk water (BW), first-draw (FD) and flushed water (FW) at the tap, and principal, secondary and return loops of a hot water system. In addition, we investigated the abundance of Legionella using quantitative real-time PCR (qPCR) and Legiolert® for Most Probable Number (MPN) enumeration, and the genetic diversity of the L. pneumophila community. Multivariate analysis based on 16S rRNA-encoding gene sequences identified five major clusters consistent with section/zones and hot water network. Representatives that explained the dissimilarity (SIMPER analysis) were associated with Alphaproteobacteria (45%), Cyanobacteria (17%), Actinobacteria (14%), Planctomycetia (11%), Betaproteobacteria (6%) and Gammaproteobacteria (3%). Furthermore, temporal variations in waterborne and opportunistic pathogen populations (Legionella, Mycobacterium and Pseudomonas) were observed among the five clusters. The Legionella population was dominated by L. pneumophila and identified as serotype 1 by agglutination and genomic analysis. FD samples from taps showed the highest counts of Legionella (=1457 MPN/100mL) with a decrease in disinfectant residual (0.04 mg Cl2/L) and temperature (21.1°C). Legionella numbers tended to decline (290 MPN/100mL), and the temperature (46.2°C) and disinfectant residual (0.12 mg Cl2/L) increased with flushing. In this study water quality was found to deteriorate due to stagnation and continuous temperature monitoring revealed inconsistent flow patterns in the hot water system, which produced temperature zones that differed significantly from the hot water tank. Overall, these results provide an ecological insight of the microbial community and the potential risk associated with Legionella.
Abstract Title: Microbial Quality Assessment of Hostel Facilities in Alvan Ikoku Federal Coll. of Ed. Owerri, Nigeria

Primary Author Block: K. S. Dike1, I. U. Offor-Emenike2, Y. E. Ezere1, M. C. Maduwuba1;  1IMO State Univ., Owerri, Nigeria, 2Alvan Ikoku Federal Coll. of Ed., Owerri, Nigeria

Abstract Body: Background: One of the major challenges in managing tertiary education in Nigeria is the inability of government to adequately provide and maintain accommodation for a teeming population of students who successfully gain admission into various programmes. Consequently, rooms designed for 4 persons now houses ten. The poor sanitary condition of the existing few hostel facilities has thus become a vehicle for the transmission of pathogenic microorganisms. This work was therefore carried out to evaluate the microbial quality of students’ hostel facilities in Alvan Ikoku Federal College of Education Owerri

Methods and Results: A total of 80 samples were collected from toilets (floor, wall and seat) bathrooms (wall and floor), room (door knobs, wardrobes, windows, walls, and floor), kitchen (sink, table and cabinet) and rails in 8 hostels (4 male and 4 Female hostels). Samples were collected using sterile swab sticks. Microorganisms were isolated and enumerated following microbiological standard and characterized using a combination of cultural/biochemical and 16S rRNA/23s rRNA sequencing techniques. Antibiotic susceptibility of the isolates was performed by agar disk diffusion method. A coliform count of 6.4 X103cfu/ml was recorded from bathroom floor samples in the female hostels. Feacal coliform count of 2.0 X102 cfu/ml was obtained from toilet floor samples in the male hostels. Fungi count of 8.0 X 102 cfu/ml was obtained from the windows in the female rooms. A total of twenty five bacteria comprising seven species [Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Staphylococcus spp, Bacillus cereus, Pseudomonas aeruginosa and Salmonella typhi] and eighteen moulds comprising Aspergillus niger, Aspergillus flavus, Penicillium spp, cladosporium spp, Fusarium spp and Aspergillus fumigatus were isolated. Escherichia coli (25%) was the most predominant organism while Bacillus cereus was the least (6.25%). Aspergillus fumigatus was the most dormant mould isolated from the samples. Exactly 80% of the bacteria isolates were resistant to cefuroxime, 73% to ceftazidine, 60% to ampicillin, 6.7% to ciprofloxin, ofloxacin and nitrofurantoin. Multi drug resistance was observed in 8 (32%) of the isolates. Conclusion: The isolation of pathogens (bacteria and fungi) and multidrug resistant bacteria isolates in these hostel facilities represents a potential risk to students in disease transmission.
Abstract Title: Turning Plastic Waste Into Added Value Materials
Primary Author Block: I. Radecka, B. Johnston, D. Hill, M. Kowalczuk; Univ. of Wolverhampton, Wolverhampton, United Kingdom
Abstract Body:
Mountains of plastic wastes are buried in landfill sites, also millions of tonnes of plastic waste leaks into our oceans each year. This continues to pose a growing challenge for authorities around the world [1,2]. Naturally-occurring bacterial polymers have an enormous potential as they can be produced from renewable resources under well controlled conditions. Polyhydroxyalkanoates (PHAs) are a group of biocompatible, environmentally neutral, biodegradable plastics that can be produced by certain bacteria [1]. One of the factors limiting the mass usage of PHAs is the high cost of the carbon sources required by microbial cells and the expensive processing requirements to extract and develop stable PHA structures in comparison to the wide range of petrochemical plastics currently in use [2]. Attempts are therefore being made to find new ways in which to increase the rate and efficiency of microbial synthesis of bioplastics. The objective of this work was to develop a viable biotechnological process in which a waste plastic can be turned by bacteria into added value materials. This study introduced the novel use of oxidized polyethylene wax (O-PEW) and non-oxidized polyethylene wax (N-PEW) substrates carbon sources for bacteria [3]. These waxes were then fed to the bacteria to make PHAs. The bacterial strain of Cupriavidus necator H16 was selected for the study as it been reported to utilize a wide range of carbon source including fatty acids for PHA production. C. necator H16 was grown for 48 hours in nitrogen rich or nitrogen-limited media that were supplemented with O-PEW or N-PEW [5]. Under those conditions the accumulation of PHAs varied from 20% to 40 % (wt / wt) of dry biomass in both media. All bacterial polymers produced were analysed using NMR, GPC and they were further evaluated with ESI-MS/MS. Analysis revealed that the PHAs obtained contained 3-hydroxybutyrate and up to 3 mol % of 3-hydroxyvalerate as well as 3-hydroxyhexanoate co-monomeric units [3, 4]. It can be concluded, that both waxes could be a promising carbon source for PHA production. Obtained data provide a strong ‘proof of concept’ that the addition of PE waxes to the microbial growth medium can have an influence on the structure of bacterial polyesters made and their chemical properties. This study demonstrates that PHA producing bacteria can contribute to the solution of the problem of disposal of manufactured plastics.
Thermochemical (TC) biomass conversion processes such as pyrolysis are promising technologies for the sustainable production of fuels and chemicals from lignocellulose. However, these processes generate a considerable amount of organic-rich, heterogenous, highly toxic wastewater streams, which are challenging to convert via standard wastewater treatment approaches without a priori detoxification strategies, as well as representing a process cost for the TC biorefinery. To adapt the biological funneling concept for valorizing waste carbon in a TC process, we first comprehensively characterized a range TC wastewater streams from pilot-scale operation, which led to identification and quantification of around 200 compounds including aldehydes, ketones, acids, aromatics, and sugars at near-complete mass closure. Based on the compositional analysis, we employed the robust and metabolically versatile microbe, Pseudomonas putida, as a biocatalyst for valorization of these streams. However, the extreme toxicity of these streams hampers P. putida growth and carbon utilization. Analysis of the chemical toxicity of the TC streams reveals that aldehydes are by far the most inhibitory compounds in these streams to P. putida. Multi-omics and biochemical analyses of P. putida grown in a TC wastewater streams suggest that protein damage is one of the key components of toxicity. To overcome acute protein damage under TC wastewater chemical stress, we engineered the native protein quality control machineries in a genome-reduced P. putida strain (EM42). The engineered strain improves the tolerance towards multiple TC wastewater samples in some cases up to 200-fold. The engineered strain can utilize TC wastewater carbon at an industrially process-relevant concentration without a priori detoxification as its sole source of carbon and energy. As an initial proof-of-concept, we demonstrate that the engineered strain can produce polyhydroxyalkanoates as well. When coupled to other metabolic engineering advances, such as expanded substrate utilization, this study enables new avenues of biological conversion of biomass-derived waste streams via an aerobic, engineered P. putida monoculture.
Abstract Title:
Direct Detection & Quantification of Microbial Contamination Within Home and Personal Care Products

Primary Author Block:
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Abstract Body:
Background: Home and personal care product (HPCP) industries use antimicrobial preservatives to prevent bacterial growth. HPCP formulations consist of proteins and varied carbon sources that facilitate microorganism growth. Early detection during manufacture is thus key to prevent consumer exposure to objectionable organisms such as intrinsically preservative-tolerant Pseudomonas aeruginosa and Burkholderia cepacia complex bacteria. We aimed to detect bacterial contamination directly from HPCPs and explore: (i) if intact metagenomic DNA can be extracted from HPCPs; (ii) the metataxonomic bacterial diversity associated contamination in comparison to routine cultivation-based monitoring; (iii) optimal extraction methods for detecting key contaminant species in a range of HPCPs. Methods: 14 contaminated industrial HPCPs of varied ages were subjected to total DNA extraction using an automated, kit-based method. Bacterial diversity was examined by 16S rRNA gene pyrosequencing and P. aeruginosa specifically detected by oprL and phzS gene-specific PCRs. Optimisation of the DNA extraction and PCR was evaluated across 3 types of HPCP spiked with B. cepacia, P. aeruginosa and Staphylococcus aureus. Organism specific quantitative real-time (B. cepacia rpoD; P. aeruginosa gyrB) and nested PCRs (S. aureus rRNA intergenic spacer region V-VI) were used to determine the detection limits of the cultivation independent procedures. Results: Metagenomic DNA was extracted and amplified using a 16S rRNA gene PCR from 12/14 contamination incident samples; bacterial diversity analysis showed that Pseudomonas was the dominant organism in 7/10 products subject to pyrosequencing. Correlation of culture and culture-independent methods was also observed for samples containing P. aeruginosa and Enterobacter sp. DNA was extracted from all artificially contaminated HPCPs, but were not amplifiable in the case of lotion products. qPCR detected ≥ 103 CFU/ml B. cepacia and P. aeruginosa, while nested PCR detected ≥ 103 CFU/ml S. aureus. Conclusions: Taxonomic profiles were successfully defined using DNA extracted from HPCPs and showed that one contaminant species predominates. Molecular detection methods can be used to detect specific contaminant organisms in a range of HPCPs.
A facultative anaerobic, Gram-negative, non-spore forming, rod-shaped marine bacterium was isolated from a biofilter from a seawater recirculating aquaculture system (RAS) in South Korea. The isolate designated as RR4-38T showed 16S rRNA sequence similarity under 95 % compared to the other genera Ulvibacter (similarly 95 %), Aureitalea (94.7 %), Gilvibacter (93.3 %), Aureisphaera (93.2 %), Jejudonia (93 %), Winogradskyella (93 %), Bizonia (92.6 %) and Flavivirga (92.5 %) which belong to the family Flavobacteriaceae. The strain RR4-38T showed growth under the presence of 1-4% NaCl, at 10-30 °C and pH 5.0-8.0, with optimum growth at 2% NaCl, 27 °C and pH7.5. Catalase and oxidase are negative. Strain RR4-38 was positive for hydrolysis of esculin, gelatin and enzyme activity of Leucine arylamidase and naphtol-AS-BI-phosphohydrolase, but negative for reduction of nitrate, glucose assimilation, alkaline phosphatase, esterase (C4), lipase(C14), α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosidase, α-mannosidase and α-fucosidase activities. Phylogenetic and chemotaxonomic characteristics with distinctive phenotypic properties revealed that strain RR4-38T is a novel species in a novel genus, for which the name Pukyongia salina gen. nov., sp. nov. (type strain RR4-38T=RR4-38T) is proposed.
Abstract Title:
High-Throughput Detection and Enrichment of Candidatus ‘Methanoperedens Nitroreducens’-Like Archaea from Different Environmental Niches

Primary Author Block:
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Abstract Body:
The newly discovered Candidatus ‘Methanoperedens nitroreducens’ (M. nitroreducens), which mediates nitrate-dependent anaerobic methane oxidation, is an important microorganism in linking carbon and nitrogen cycles. In order to explore the diversity of M. nitroreducens-like archaea in various environmental niches with advanced high-throughput sequencing, new primers based on alpha subunit of methyl-coenzyme M reductase (mcrA) gene was designed. The PCR results demonstrated that the new primers could effectively detect M. nitroreducens-like archaea from an enrichment culture dominated by M. nitroreducens as well as samples collected from a natural freshwater lake and a full-scale wastewater treatment plant (WWTP). By using the high-throughput sequencing, more than 30,000 M. nitroreducens-like sequences were obtained. Phylogenetic analysis of these sequences along with published sequences showed that M. nitroreducens-like archaea could be divided into three sub-branches (named as Group A, Group B and Group C in this study). Clear geological difference was observed, with Group A and Group B dominating samples in Queensland (Australia) and in European ecosystems, respectively. Further quantitative PCR revealed that the M. nitroreducens-like archaea was more abundant in WWTP than the freshwater lake. After 3 years’ enrichment of mixed inoculum of WWTP and freshwater lake in a bioreactor, the nitrate removal rate reached 32 mg N/(L•d), confirming the M. nitroreducens-like archaea detected has the real methane oxidation ability. The study provided a large number of sequences for M. nitroreducens-like archaeal communities, thus expanded our understanding on the ecological distribution and diversity of M. nitroreducens-like archaea.
Identification of Panninobacter Phragmitetus and Novosphingobium Aromaticivorans As Novel Thermotolerant Bacteria

Primary Author Block:
L. Bonsack, C. Brown, Y-F. Lu; Westmont Coll., Santa Barbara, CA

Abstract Body:
Classification of bacteria and taxonomy are important components that forward scientific discovery and disease prevention. Thermophilic bacteria can survive and thrive in higher temperatures and extreme conditions, like those of hot springs. Thermophiles have thermostable enzymes that are relevant for industrial and research technologies. In this study, water samples were harvested from the Montecito hot springs in order to discover, isolate, and characterize thermophilic microbial species. Samples were purified to obtain pure cultures in a 55ºC temperature-controlled environment. Genomic DNA was extracted and the species was identified by 16S rRNA sequencing. Two types of novel bacteria were identified and characterized: Pannonibacter phragmitetus and Novosphingobium aromaticivorans. However, the growth rate of these two bacteria species is lower in 55ºC compared to the growth in 37ºC, suggesting these two species are thermotolerant. Both of these bacterial species are clinically relevant and have not previously been identified in the literature as thermotolerant before this study. The thermotolerant finding of these two bacteria and their locations in nature have furthered the scientific understanding of these two species and could lead to possible future biotechnological applications.
Effects of NaCl on Microbial Communities and Metabolites During Fermentation of Korean Traditional Doenjang

B. Chun, K. Kim, S. Jeong, C. Jeon; Chung-Ang Univ., Seoul, Korea, Republic of

Four doenjang samples with different NaCl concentration (9, 12, 15, and 18%) were prepared in triplicate and their microbial communities and metabolites during the entire fermentation period were analyzed to compare their fermentation features using Illumina MiSeq and 1H-NMR approaches, respectively. The pH profiles were relatively similar regardless of NaCl concentrations. However, the pH values in doenjang with 9% NaCl were a little lower during the early and middle fermentation periods, but they increased to the higher pH values than those in doenjang with 12%, 15%, and 18% NaCl during the late fermentation period. The microbial communities in low NaCl doenjang changed more dynamically as compared to those in high NaCl doenjang during fermentation. Bacterial community analysis based on 16S rRNA gene sequences revealed that Bacillus, Staphylococcus, and Clostridium identified as dominant species in all doenjang samples. Bacillus and Staphylococcus were identified more abundantly in high NaCl doenjang, especially during late fermentation period, while Weissella and Clostridium were identified more abundantly in low NaCl doenjang, which might be related with their low pH values during the early fermentation period. Lactic acid bacteria such as Tetragenococcus and Lactobacillus were also present more abundantly in low NaCl doenjang during late fermentation period. Fungal community analysis revealed that Aspergillus, Penicillium, and Microascaceae identified as dominant species in all doenjang samples. Mucor was identified more abundantly in 12% and 15% NaCl doenjang during entire fermentation period. Debaryomyces and Aspergillus were present more abundantly in low NaCl doenjang during middle and late fermentation periods. Microascaceae and Penicillium were identified more abundantly in high NaCl doenjang during late fermentation period. The metabolic analysis revealed that organic acids were more produced in low NaCl doenjang, which might be correlated with their low pH values during the early fermentation period. However, the amino acid profiles were relatively similar regardless of NaCl concentrations. These results revealed the fermentation properties of the Korean traditional doenjang with different NaCl concentrations during entire fermentation period, contributing to the production of high-quality doenjang.
Abstract Title:
Effects of Fermentation on the Nutritional and Anti-Nutritional Components of Cooked/Boiled Water Melon (Citrullus Lanatus) Seed
Primary Author Block:
O. A. Makinde, D. O. Adejoro, V. O. Odubanjo, A. S. Ajayi; Adekunle Ajasin Univ., Akungba Akoko, Nigeria

Abstract Body:
Background: A variety of fruits and vegetables are consumed in Nigeria on a daily basis, and they form an integral part of our diet. However, most times only the fleshy pulp of these fruits are consumed leaving the seed and the rind. Fruits have high vitamin, mineral, fibre, phytochemical and antioxidant in diets of many Nigerians especially the seed and rind which most times are discarded due to ignorance of the nutritive value and their curative advantages, lack of proper storage facilities, poor distribution, rising cost of fruits, poor accessibility and affordability (Tindall, 2004). Methods: The effects of processing (heat treatment and fermentation) on the proximate composition and anti-nutrient components of water melon (Citrullus lanatus var. lanatus) seeds were investigated. It was carried out by boiling some of the seeds for 3h, with some seeds wrapped in clean plantain (Musa sapientum var. paradisiaca Linn.) leaves and allowed to ferment for 96h. Microbial analysis was carried out daily on the fermenting seeds. Results: Results of proximate analysis revealed a significant difference (p<0.05) in contents of protein, crude fat, ash, moisture content and carbohydrate of the raw, cooked and fermented seeds while there was no significant difference in the crude fibre of the raw and cooked seeds. Protein, crude fat and carbohydrate ranged from 8.55-13.14%, 4.64-9.76% and 49.78-60.29% respectively with the highest values in fermented seeds while the raw had the least. The anti-nutrient investigation showed that saponin was absent in raw water melon seed but present in the corresponding cooked and fermented seeds; flavonoid was present in all the three samples; alkaloid was absent in all the three samples; tannin was present only in the raw seed but absent in the cooked and fermented seed. Nine bacterial genera tentatively identified as Pseudomonas, Staphylococcus, Bacillus, Lactobacillus, Micrococcus, Proteus, Pediococcus, Klebsiella and Serratia with one fungal genera identified as Aspergillus were recovered from fermenting water melon seeds. The pH, titratable acidity and temperature of the seeds at the end of fermentation were 8.02, 0.20 and 28°C respectively. Conclusions: It was concluded that cooking and fermentation have direct relationships on proximate and anti-nutrient compositions. It is concluded that cooked and fermented water melon seeds could be a formidable source of nutrients with potential industrial and medical applications.
Abstract Title:
Pre-Pilot Fed-Batch Fermentation of Rhodococcus Rhodochrous Dap 96253 with Emphasis on Antifungal and Delayed Fruit Ripening Properties

Primary Author Block:
M. de la Croix, K. Cannon, S. A. Crow, Jr, G. E. Pierce; Georgia State Univ., Atlanta, GA

Abstract Body:
Background: Rhodococcus is a Gram-positive, aerobic, non-pathogenic bacterium ubiquitous to soil. Rhodococcus strains have been used for over 70 years in industrial fermentation to produce a variety of pharmaceutical grade products. Induced cells of R. rhodochrous strain DAP 96253, have been used successfully to delay ripening in climacteric fruits and also as a biocontrol agent of select fungi. These fungal control and fruit preservation methods play an important role in agricultural post-harvest production and prevent massive loss of inventory due to spoilage. Methods: Induced cells, obtained from a two-phase fed-batch fermentation process, were utilized to determine the effectiveness of immobilized R. rhodochrous DAP 96253 cells as a contact-independent antifungal catalyst for use with post-harvest strawberries. Cardboard inserts holding the strawberries in clamshells were sprayed with an immobilized whole-cell edible fruit wax and stored at 5°C. In addition, contact-independent delayed fruit ripening of bananas was conducted using immobilized whole-cell paste in an enclosed 4L container for eleven days at various concentrations. Bananas and whole-cell paste was stored at 25°C during the extent of the experiment. Results: Non-treated strawberries showed slight mold development on day six and by day eleven showed significant Aspergillus spp. mold. Treated strawberries showed only slight mold growth following eleven days of storage (Figure). Treated bananas showed a notable extension of ripening, while control bananas showed significant deterioration. Conclusions: Induced cells of R. rhodochrous DAP 96253 clearly showed the ability to inhibit the growth and germination of phytopathogenic Aspergillus spp. on strawberries and acted to delay ripening of bananas in a contact-independent setting. Pre-pilot fed-batch fermentation allows to produce induced cells of R. rhodochrous DAP 96253 in large quantities for future large-scale commercialization.
Session Title: AES14 - Detection, Characterization and Source-Tracking of Environmental Microbes: Isolation of Microbes from the Environment
Session Start Date Time: 6/10/2018 12:45:00 PM
Session End Date Time: 6/10/2018 2:45:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 6201
Poster Board Number: SUNDAY - 937

Abstract Title:
Evaluation of A Multiple Regression Modeling Approach to Identify Patterns of Fecal Pollution in the Tuckasegee River Watershed in Western North Carolina

Primary Author Block:
P. D. Downing, C. S. Morgan, K. K. Hall; Western Carolina Univ., Cullowhee, NC

Abstract Body:
Surface waters that are listed on impaired waters (303d) lists due to pathogen contamination pose a significant environmental and public health burden. The need to address these through the Total Maximum Daily Load (TMDL) process has resulted in the development of methods to successfully identify sources of fecal pollution. Advantages of these developed methods include their ability to characterize fecal pollution sources and their cost effectiveness to help maximize available resources to improve water quality. However, the ability of these methods to effectively and universally identify sources of fecal pollution requires further evaluation. Current TMDL methods rely on a watershed approach to identify stressors and monitor remediation efforts, yet effective Best Management Practices (BMPs) implementation must consider watershed dynamics. The objective of this research was to assess the usefulness of a previously described multiple regression modeling approach to characterize watershed dynamics and prioritize streams for remediation efforts using the Tuckasegee River watershed in Western North Carolina as an example. Three multiple regression models were developed using chemical and microbial water quality data collected monthly from Scott and Savannah Creeks from May 2016 to April 2017. Model 1 included all water quality parameters and Model 2 included statistically significant parameters identified by stepwise regression. The water quality variables included in Model 3 were statistically significant as identified by canonical discriminant analysis. These models were applied to determine if they correctly classified land use patterns and subsequent levels of fecal pollution throughout the watershed and within individual creeks. The developed models were statistically significant but with low r2 values (Model 1 r2=0.04, Model 2 r2=0.03, Model 3 r2=0.03). Only model 2 successfully classified land use at the watershed level (p=0.008, r2=0.02). No model successfully classified land use at the creek level. These results suggest there is variability in the extent and sources of fecal pollution within the Tuckasegee River watershed as it relates to land use patterns. Considering these sources of variability using this statistical approach may be a useful tool in setting priorities and identifying streams that should be treated in independent TMDLs in order to develop and implement BMPs that successfully remove water bodies from 303d lists.
Abstract Title:
Isolation and Study of Cellular Components of Aerobacillus Polymyxa and Bacillus Megaterium Along with its Comparison in Soil Layers

Primary Author Block:
W. Shahid; Jinnah Univ. for Women., Karachi, Pakistan

Abstract Body:
Objectives: The main objective of this research was to isolate and to study the cell morphology and biochemical reactions of Aerobacillus polymyxa and Bacillus megaterium along with its habitat either in deep soil or aerobic soil. Background: Aerobacillus belongs from the family of “Penibacillus polymxa” and bacillus megaterium belongs from “Bacillaceae” family. These two organisms are gram positive, non-pathogenic bacteria found in soil that helps in nitrogen fixation. They both are equally important today but the main aim of this research was to isolate them from the soil due to the characteristic importance of A. polymyxa to produce antibiotic and to remove biofilm formation, while B. megaterium is also a good source of producing industrial proteins due to its larger size than any other microorganisms. Isolating these organisms was difficult due to the high chances of growth of bacillus subtilis and other organism that are commonly found in soil and environment. Methodology: Total 16 samples were collected from aerobic & anaerobic soil for A. polymyxa and 16 samples were collected from aerobic, anaerobic, water and milk for B. megaterium. The samples were cultured on Tryptone Beef Extract medium (TGB) and Nutrient agar (NA) for four days. TGB media is specific for A. polymyxa. Plates were then incubated at 37°C and were allowed to grow for 4 days. Plates were checked daily in order to check the growth. 5th day plates were observed for cultural morphology. Each kind of colonies from the plates was picked to observe gram reactions of the organisms. Colonies were also observed for biochemical reactions. Results: Out of 16 samples, 9 samples showed positive results for the colonies of A. polymyxa. Also, 12 samples out of 16 samples showed positive results for B. megaterium, further confirmed by biochemical reactions. Conclusion: This research sums up the isolation method of A. polymyxa and B. megaterium, its habitat and its cell morphology. It also showed that they are mostly present in deep roots soil where they play an important role in fixing nitrogen. These organisms can be used widely in industries due to the activity involved in bioflocculation and biopreservation of foods. Growing these two microorganisms in laboratory according to their growth conditions can be helpful for various purposes.
Abstract Title:
Novel Enterobacteriaceae Produces Antibiotic Effective against Eskapes

Primary Author Block:
K. H. McCain, D. A. Davis; Wingate Univ., Wingate, NC

Abstract Body:
Background: The emergence of pathogens that have gained resistance to antibiotics has led to a great need for the discovery of novel antibiotics. Crowd sourcing programs like the Small World Initiative were created to help supplement this lack of development by isolating bacteria from microbially diverse soil samples and screening them to identify those capable of antibiotic production effective against ESKAPE pathogens. Given the numerous and diverse microorganisms in soil, it was hypothesized that novel antibiotic producing bacteria would be isolated from nutrient rich soil in a residential yard. Methods: Antibiotic producing bacteria were isolated from a sandy clay loam soil sample in Monroe, North Carolina. Nineteen morphologically varied isolates were screened using in vivo plate assays against the safe relatives of the ESKAPE pathogens (Enterococcus raffinosus, Staphylococcus cohnii, Escherichia coli, Acinetobacter baylyi, Pseudomonas putida, Enterobacter aerogenes, and Bacillus subtilis). One isolate, 24, expressed the ability to produce potent antimicrobial compounds capable of inhibiting the growth of all 7 ESKAPE pathogen safe relatives. Isolate 24 was characterized morphologically, and its biochemical capabilities, including its ability to hydrolyze complex nutrients, ferment a variety of sugars, and utilize inorganic electron acceptors were determined. An attempt was made to obtain a preliminary identification through sequencing of the 16S rRNA gene using universal PCR primers. To obtain a more definitive identification of the isolate and its genetic makeup, the full genome sequence was obtained using Illumina MiSeq. The genome was assembled, analyzed, and annotated using the Galaxy open source application. Results: Isolate 24 is a Gram negative bacillus shaped bacterium with a demonstrated ability to ferment lactose, mannitol, and utilize nitrate as an electron acceptor. Preliminary identification indicates that isolate 24 is of the Class Gammaproteobacteria, and the Family Enterobacteriaceae, however genus information was inconclusive. Full genome analysis indicates there are 4,660,524 base pairs in the isolate’s genome and 4288 genes. Conclusions: This data indicates that isolate 24 is a novel Enterobacteriaceae that produces antibiotics effective against ESKAPE pathogens. Further exploration of the genome will provide accurate taxonomic information on this proteobacterial strain and indicate the genes responsible for the potent antibiotic[s] produced by this organism.
Abstract Title: Maldibase: A Publ. Web Application for the Identification of Microbes by Analysis of Protein Spectra Generated by Maldi-Tof

Primary Author Block: M. G. LaMontagne, A. K. Kothapalli, S. Sadu, N. Yenegro, K. K. Konathala, P. K. V. Buddharaju; Univ. of Houston Clear Lake, Houston, TX

Abstract Body: Background: Matrix-assisted laser desorption ionization - time of flight (MALDI-TOF) provides a rapid method of microbial identification. Commercially available systems allow strain-level identification of microbes in minutes for pennies an isolate; however, these systems use proprietary software and databases. These databases were developed primarily for clinical applications, which limits the application of this proteomics approach. Here we present MALDIbase (https://uhcl.shinyapps.io/taxonomy-databank/), a public, web-based system and database. The system uses Rshiny to create a graphic user interface to R, an open source programming language and software environment for statistical computing and graphics. The application runs MALDIquant, an R package, to allows users to process spectra generated by MALDI-TOF. This processing involves selecting parameters for peak detection and aligning. Users can export aligned peaks and store these spectra in the cloud.

Methods: For microbial identification, the application runs the J48 machine learning algorithm in RWeka. To evaluate this algorithm and the application overall, we processed 125 spectra generated in two MALDI-TOF runs from bacteria isolated from various environments and microbiomes by students in an undergraduate microbiology laboratory class. We wrote a script to select optimal peak processing parameters. This script randomly selects input parameters and repeatedly tries to align and select peaks. We selected the combination of parameters that maximizes the percentage of peaks shared between replicate runs of the same sample. Results: This optimization yielded parameters that detected 411 - 467 peaks for the two runs; 71 - 90 % of these peaks were shared by duplicate spectra generated from the same isolate. Classification at the species level, using the J48 algorithm, succeeded for 92 % of spectra overall. Classification success reached 100 %, for species with >5 spectra per species. For example, 27/27 and 18/18 of spectra generated from E. aerogenes and E. coli isolates respectively were successfully identified. Conclusions: This suggests classification success will improve steadily as the database grows and underscores the value of a public database of spectra that accepts contributions from the community.
Session Number: 461
Session Type: Poster Talk
Session Title: Microbial Interactions in the Urban Environment
Session Start Date Time: 6/10/2018 1:45:00 PM
Session End Date Time: 6/10/2018 2:35:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 8724
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Robert Sowah; 1
Abstract Body:
Staphylococcus aureus Prompts Escherichia coli Survival Under Dry Conditions: A Potential Threat from the Viewpoint of Nosocomial Infections

Primary Author Block:
T. Okubo1, T. Shimoda1, S. Nakamura2, R. Yano1, J. Matsuo1, H. Yamaguchi1; 1Hokkaido Univ., Sapporo, Japan, 2Juntendo Univ. Graduate Sch. of Med., Tokyo, Japan

Abstract Body:
Background: High-touch places are a threat referred to “hot spots” facilitating the spread of hand-attached bacteria in hospitals, responsible for nosocomial infection. Therefore, both ATP and stamp methods have developed to evaluate the high-touch places with bacterial contamination quickly. Meanwhile, our previous studies showed unintelligible a prominent gap between the amounts of ATP and bacterial colony in hospital floors maintaining dry conditions, presumably underlying unfitted method or unknown bacterial dynamics under dry. Hence, we evaluated bacterial dynamics on dry-floor materials at distinct temperatures by the ATP and stamp methods with accurate monitoring bacterial numbers. Methods: Staphylococcus aureus ATCC 29213 and Escherichia coli ATCC 25922 were separately suspended, and mixed together (or not) in LB broth. The 20μl of each solution (5.0 × 10^5 CFU/spot) were then spotted on the flooring materials (3cm × 3cm). They were then dried and incubated at distinct temperatures (room, 30°C, 37°C) up to 11 days. After incubation, dried spots were wiped with cotton swabs, and suspended into PBS. The amounts of both bacteria was evaluated by spreading out on Mannitol salt agar and MacConkey agar, expressed as CFU per spot. Furthermore, the spots were visualized by the observation with SEM. The amounts of ATP on each spot was also monitored by Clean-Trace Luminometer (3M, USA). Results: As expected, in the dry case of single suspension, S. aureus endured with a gradual decrease of CFU over time, although E. coli rapidly died until 4 days. Meanwhile, in the dry case mixed with S. aureus, E. coli survived more longer time; it tended to come stronger under low temperature. However, such bacterial dynamics could not be detected by the ATP measure, regardless of single or mixed. SEM revealed no morphological differences of both bacteria between single and mixed cases. Conclusions: We concluded that S. aureus prompts E. coli survival under dry conditions, responsible for a potential threat from the viewpoint of nosocomial infection. Also, ATP measure likely discouraged us from accurately implementing hospital cleanliness against nosocomial infection.
Abstract Title:
Widely Used Disinfectants Can Promote Antibiotic Resistance

Primary Author Block:
M. Kim1, M. R. Weigand2, S. Oh3, J. K. Hatt1, R. Krishnan1, U. Tezel4, S. G. Pavlostathis1, K. T. Konstantinidis1; 1Georgia Inst. of Technology, Atlanta, GA, 2CDC, Atlanta, GA, 3Nanyang Technological Univ., Singapore, Singapore, 4Bogazici Univ., Istanbul, Turkey

Abstract Body:
Background: While misuse of antibiotics has clearly contributed to the emergence and proliferation of resistant bacterial pathogens with major health consequences, it remains less clear if the widespread use of disinfectants such as quaternary ammonium compounds (QAC) has contributed to this problem. Here, we provide evidence that exposure to benzalkonium chlorides (BAC), a widely used member of QAC, co-selects for antibiotic-resistant bacteria, and describe the underlying genetic mechanisms.

Methods & Results: BAC-fed bioreactors inoculated with river sediment selected for several bacterial taxa, including the opportunistic pathogen Pseudomonas aeruginosa, that were more resistant to several antibiotics compared to their counterparts in a control (no BAC) bioreactor. Metagenomics analysis of the bioreactor microbial communities, confirmed by genetic manipulations of derived isolates, suggested that integrative and conjugative elements encoding a BAC efflux pump together with antibiotic resistance genes were mainly responsible for these results. Further, exposure of the P. aeruginosa isolates to increasing concentrations of BAC selected for mutations in pmrB (polymyxin resistance) and physiological adaptations, which contributed to higher tolerance to polymyxin B.

Conclusions: Collectively, our results demonstrate that disinfectants can promote antibiotic resistance via several mechanisms, and highlight the need to remediate (degrade) disinfectants in non-target environments to further restrain spread of antibiotic resistant bacteria.
Abstract Title:
Glycocalyx Slime-Producing and Autotrophic Bacteria Used in Self-Healing Technology for Concrete Cracking

Primary Author Block:
Y. Park, H-S. Kim, J. Kim, S. Kang, S. Jeong, S-S. Lee; Kyonggi Univ., suwon, Korea, Republic of

Abstract Body:
Development of self-healing concrete is required for recover concrete-cracking because the technique can solve the problems in economic aspect. This study is to develop a self-healing technique for concrete-cracking during the long term by using, which can be tolerance to strong alkali environment. Glycocalyx slime-producing bacteria were selected in the previous study. Rhodobacter capsulatus(KACC 15298), Rhodobacter blasticus(ATCC 33485) were used to distinguish slime due to the carbon source change. Both strains with 50ml of each strain and 5L of each culture with two kinds of carbon source (succinate, malate). Strains incubate at 28±2°C for 30 days under light. Slime components from each bacteria strain were analyzed by high-performance liquid chromatography (HPLC), gas chromatography-flame ionization detector (GC-FID), enzyme-linked immunosorbent assay (ELISA). When strains used succinate for carbon source, R.capsulatus had 10.0 ± 0.006 mole% of neutral sugar, 1.0 ± 0.009 mole% of uronic acid and 22.2 ± 0.003 mole% of protein. R.blasticus had 9.3 ± 0.002 mole% of neutral sugar, 1.6 ± 0.002 mole% of uronic acid and 21.9 ± 0.006 mole% of protein. When strains used malate for carbon source, R.capsulatus had 8.0 ± 0.007 mole% of neutral sugar, 1.8 ± 0.006 of uronic acid and 12.5 ± 0.009 of protein. R.blasticus had 5.4 ± 0.002 mole% of neutral sugar, 1.2 ± 0.002 mole% of uronic acid and 11.3 ± 0.005 mole% of protein. Thus, both strains showed more protein and neutral sugars in slime produced using malate as a carbon source than succinate. For analysis of slime molecular weight, when each bacteria strain incubated with using succinate as a carbon source, R.capsulatus was measured as 348.3 kDa and R.blasticus as 687.9 kDa. When using malate as a carbon source, both strains had not effective molecular weight. Therefore, R.capsulatus was composed of small slime molecular weight at the same amount, molar concentration was found to form more slime than R.blasticus. Evaluation of the amount of slime production, when succinate was used as a carbon source, R.capsulatus produced 2.57 g/L of slime, R.blasticus produce 1.03 g/L of slime. When malate was used as a carbon source, R.capsulatus produced 9.54 g/L of slime, R.blasticus produced 3.74 g/L of slime. Therefore, R.capsulatus using malate as a carbon source showed the highest slime product efficiency.
Abstract Title:
Water Quality and Microbial Dynamics in A Large Building Hot Water Sys. and Managing Potential Risk Associated with Legionella

Primary Author Block:

Abstract Body:
Premise plumbing (PP) includes that portion of the drinking water distribution system (DWDS) connected via the service line to houses and other buildings. Water quality in PP is not monitored by U.S. EPA regulation with the exception of the Lead and Copper Rule. Because public health data shows that a significant fraction of the nation’s waterborne disease outbreaks are attributable to PP systems, it is important to understand the characteristics of these systems which amplify the potential public health risk relative to the DWDS. The present study was conducted in a 40-year-old large building supplied with treated chlorinated water with a main and secondary hot water network. We used physico-chemical parameters, heterotrophic plate counts (HPC), and 16S rRNA sequencing data to generate water quality profiles of the bulk water (BW), first-draw (FD) and flushed water (FW) at the tap, and principal, secondary and return loops of a hot water system. In addition, we investigated the abundance of Legionella using quantitative real-time PCR (qPCR) and Legiolert® for Most Probable Number (MPN) enumeration, and the genetic diversity of the L. pneumophila community. Multivariate analysis based on 16S rRNA-encoding gene sequences identified five major clusters consistent with section/zones and hot water network. Representatives that explained the dissimilarity (SIMPER analysis) were associated with Alphaproteobacteria (45%), Cyanobacteria (17%), Actinobacteria (14%), Planctomycetia (11%), Betaproteobacteria (6%) and Gammaproteobacteria (3%). Furthermore, temporal variations in waterborne and opportunistic pathogen populations (Legionella, Mycobacterium and Pseudomonas) were observed among the five clusters. The Legionella population was dominated by L. pneumophila and identified as serotype 1 by agglutination and genomic analysis. FD samples from taps showed the highest counts of Legionella (=1457 MPN/100mL) with a decrease in disinfectant residual (0.04 mg Cl2/L) and temperature (21.1°C). Legionella numbers tended to decline (290 MPN/100mL), and the temperature (46.2°C) and disinfectant residual (0.12 mg Cl2/L) increased with flushing. In this study water quality was found to deteriorate due to stagnation and continuous temperature monitoring revealed inconsistent flow patterns in the hot water system, which produced temperature zones that differed significantly from the hot water tank. Overall, these results provide an ecological insight of the microbial community and the potential risk associated with Legionella.
Abstract Title:
Metagenomic Insights Into the Mechanisms Underlying the Depth-Stratified Microbial Diversity Patterns in the Ocean’S Interior
Primary Author Block:
D. Tsementzi, R. Conrad, A. Mezit, L. Rodriguez R, K. Konstantinidis; Georgia Inst. of Technology, Atlanta, Georgia
Abstract Body:
Culture-independent exploration of the oceans has identified depth-stratified microbial assemblages and revealed several genomic adaptations related to the deep ocean environment. However, the number of samples available from the deep sea remains limited, as is our understanding of the underlying mechanisms for several of the depth-specific patterns observed previously. Here, we aimed to provide new insights into these issues using deep Illumina sequencing of samples from highly stratified water masses in the Gulf of Mexico at 3 stations, from surface down to 2,100m. Comparison of taxonomic profiles revealed strong clustering of populations by depth, and not by location, even when including previously determined samples from geographically distant locations. In other words, close relatives of populations recovered from certain depths were identified along the depth profiles with decreasing genetic relatedness (measured by sequence identity) at increasing depth distance from the reference population/depth. Our analysis shows that this is a gradual as opposed to step-wise decrease in genetic relatedness, driven mostly by protein adaptation to the physicochemical properties (e.g., hydrostatic pressure) of the depths considered. Finally, comparison of functional distributions revealed shifts in gene content between surface and deep ocean communities, e.g., photosynthesis, phosphate metabolism and viral proteins were enriched in the surface samples, while aromatic compound metabolism, various peptide transporters, and transposases and integrases were found enriched in the deep water samples, in accordance with previous observations. The transposase signal appears to be tied to abundant members of the SAR324 lineage (nearly 40% of identified transposases), but a large diversity of transposases was also found outside of this group, indicating that the signal cannot be entirely explained by gene hitchhiking. Moreover, the SAR324 populations are abundant in a wide depth range, but only appear to harbor large numbers of transposases in the deepest samples, indicating a selective advantage of mobile genetic elements in deep ocean environments.
Abstract Title:
Prokaryotic Species Diversity in Great Smoky Mountains Natl. Park: from 16s Rdna to Whole Genomes
Primary Author Block:
S. P. O'Connell, T. K. Carlson, L. M. Dye, K. R. Fraser, R. P. McKinnon; Western Carolina Univ., Cullowhee, NC
Abstract Body:
We have been sampling the microbial diversity of Great Smoky Mountains National Park (GSMNP) for over 15 years. Much of this work attempts to understand bacterial and archaeal distributions in unique habitats such as the rhizosphere of Eastern Hemlock (Tsuga canadensis), elk rumens (Cervus elaphus), and in stream waters and soils. Recently, the genomes of four bacteria from a stream were sequenced in order to verify their uniqueness. Over 500 cultured and 1,200 clones have been documented in our work and compared using sequences of 16S rDNA. Classifications for the species have been made using Classifier and SeqMatch tools within the Ribosomal Database Project (RDP) and 15 total phyla of bacteria and archaea have been encountered. The cultures have been dominated by Proteobacteria, Firmicutes, and Bacteroidetes with the genera Paenibacillus, Streptomyces, and Pseudomonas particularly common. The clones show a very high and diverse distribution of Acidobacteria in soils and rhizospheres as well as many genera from the Proteobacteria. Ammonia oxidizing archaea were also common. Using RDP results and phenotypic testing, four cultures including three from the Enterobacteriaceae and one from the genus Paenibacillus, were shown to be low matches to known species. These cultures had their whole genomes sequenced using an Illumina HiSeq 2500 system. Gene annotations and alignments to other sequenced genomes showed that one matched a known species (Erwinia billingiae), two were probably new genera within Enterobacteriaceae, and one was a novel species of Paenibacillus. Our work in GSMNP corroborates high biodiversity shown in GSMNP for other taxa, e.g., in salamanders, plants, and insects. It also appears that soils may be dominated by Acidobacteria, which is unusual compared to other soils around the globe. Whole genome sequencing supports our hypothesis that 16S rDNA can predict the novelty of bacteria and it gives additional tools to examine (e.g., genes for pathogenicity, phages, resistance to metals and antibiotics, transposons; and for employing unique biochemical reactions) that may give us broader insight into the biology of these organisms in situ. Such information will be of great value to Park managers and to microbiologists interested in discovering links between genes and ecology in a natural setting.
Abstract Title:
Characterization of Ship Microbiomes and Port Microbial Communities in the Great Lakes
Primary Author Block:
S. M. Techtmann, R. B. Ghannam, L. G. Schaerer, T. M. Butler, M. Breneman; Michigan Tech Univ., Houghton, MI
Abstract Body:
Ports throughout the world have been impacted by anthropogenic activity and invasive species. These locations are constantly being exposed to new inputs through ship traffic, industrial activity, and ballast water exchange. Ships carry with them a diverse microbial community. The microbiome of a vessel may to some extent reflect the waters through which it has passed. Many ports undergo dramatic seasonal changes, that can alter the composition of the microbial communities in these ports. We investigated the impact of seasonal change and ship traffic on the microbial community composition of three ports in the Great Lakes. Samples were collected from Duluth-Superior, Green Bay, and the Keweenaw Peninsula in Fall of 2016 and Summer of 2017. Samples were also collected from the boats used for sampling to identify the microbiome of the vessels and how the waters through which these vessels transits impact the vessel microbiome. The microbial community composition of these samples was profiled using 16S rRNA sequencing. The environmental conditions in these ports changed marginally between these two sampling times with temperature changing the most substantially between sampling events. The overall microbial community composition was significantly different between these three ports based on PERMANOVA analysis. Ports on the same body of water had distinct microbial communities. Certain microbial groups are substantially enriched in particular ports relative to others. The microbial community composition changes dramatically in these ports between seasons. Bilge water and ship surfaces were also sampled to investigate the impact of ship traffic in the dispersal of microbes from one location to another. Our results also suggest that boats can carry indicator species from one port to others. For example, Microcystis was present at an average of 8.6% of the microbial community in Green Bay, and less than 0.01% of the community in the other ports. Bilge water was sampled from a boat that was in Green Bay and transported to Duluth. After sampling in Duluth, Microcystis were present at more than 30 times the abundance in Duluth waters. This indicates that microbes can be transported from one location to another via boat traffic, suggesting that ship traffic has the potential to contribute to microbial dispersal in aquatic environments.
**Abstract Title:**
Phytobiome: What Does Domestication Do to Beet Associated Bacteria?

**Primary Author Block:**
M. Sikora, S. Szymańska, K. Kurnik, E. Deja-Sikora, M. Skorupa, A. Tretyn, K. Hryniewicz, J. Tyburski, M. Golebiewski; Nicolaus Copernicus Univ., Torun, Poland

**Abstract Body:**
Background: Beet is a widely planted crop with its wild ancestor still growing on seashores in Europe. This gives us a unique opportunity to study influence of domestication on plant microbiome composition and its sources - soil as opposed to seeds. We hypothesized that i) beet microbiome composition would depend on plant genotype but certain organisms would be common due to their importance for the host and evolutionary history - these would be transmitted vertically via seeds, ii) different organisms would be recruited from soil depending on genotype and iii) the same organisms would be prevalent in metagenomic and culture-based analyzes.

Methods: To compare microbiomes of sugar beet (cv. 'Huzar') with wild beet (B. vulgaris ssp. maritima) grown in garden soil, we amplified V3-4 fragments of 16S rRNA genes from seeds, plants and soil, converted them to Illumina libraries and sequenced the libraries on MiSeq. Bioinformatic analyzes were performed with dada2 (an R package) and Mothur, and community analysis was done with vegan and phyloseq in R. Additionally, at the end of the experiment bacteria were isolated from roots and identified by means of 16S rDNA sequencing.

Results: Sugar beet and wild beet microbiomes were indeed different, however their common feature was high share of Proteobacteria. Only a small portion of organisms originated from seeds, and there was large overlap between endophytic and soil communities. Bacterial isolates turned out to represent various phyla and constituted only a small fraction of diversity recovered with metagenomics.

Conclusions: In conclusion, domestication seems to have changed not only beet’s genetic landscape, but also its microbiome. Soil has the largest impact on beet microbiome, however genotype modulates to some extent the spectrum of recruited organisms.
Abstract Title:
Microbial Communities in Accelerated Low Water Corrosion on Marine Sheet Piling
Primary Author Block:
H. C. Phan1, S. A. Wade1, L. L. Blackall2; 1Swinburne Univ. of Technology, Hawthorn, Victoria, Australia, 2Univ. of Melbourne, Parkville, Victoria, Australia
Abstract Body:
Major infrastructure such as ports and harbours, located on the coasts throughout the world typically contain critical metal components/structures immersed in marine waters. These structures can often suffer from severe degradation at the low tide water level due to a phenomenon known as accelerated low water corrosion (ALWC), which is a form of microbiologically influenced corrosion. We obtained samples of the orange-coloured corrosion material (also known as “orange bloom”) commonly associated with ALWC from steel sheet piling at a field test site in a seaside harbour on Port Philip Bay, Victoria, Australia. These samples included a combination of corrosion by-products and microbial biofilm/biomass, which were evaluated for the presence of sulfate-reducing, acid producing and iron-related bacteria using commercial test kits. In general, positive confirmation of the presence of each of these bacterial types was found from these test kits. The microbes in the outer and inner layers of the orange bloom on different steel types, and from adjacent seawater were also studied by pure culture isolation and by metabarcoding of the V4 region of the 16S rRNA genes and analysis by the QIIME pipeline using the NCBI database as a reference. According to molecular analyses, Deltaproteobacteria (which includes many sulfate reducing bacteria) were abundant in the inner of the orange bloom compared to the outer, while sulfur oxidisers were abundant in the outer layer compared to the inner. The microbial communities varied more by the location in the orange bloom (i.e., inner or outer layer) than by the steel sheet type. Additionally, the adjacent seawater had significantly distinct microbial communities (p < 0.05) compared to the orange bloom. While more than 100 pure culture isolates were obtained from one orange bloom sample by aerobic or anaerobic incubation, there was little correlation between the results of isolation and the species identified by metabarcoding. This work provides new information on the complex microbial communities associated with ALWC and has generated an ALWC microbial culture collection which will be used in subsequent laboratory-based corrosion tests. This information will be used to assist in the development of future strategies to mitigate this major corrosion problem.
Session Number: 500  
Session Type: Rapid Fire

Session Title: The Wonderful World of Agricultural and Environmental Microbiomes  
Session Start Date Time: 6/10/2018 4:30:00 PM  
Session End Date Time: 6/10/2018 5:15:00 PM  
Session Primary Track: Applied and Environmental Science  
Abstract Control Number: 8876

Abstract Title: The Fine Level Diversity, Dynamics and Function of the Cocoa Bean Fermentation Microbiome: A Sys. to Study and Model Microbial Communities

Abstract Body:

Background: Cocoa bean fermentation is a crucial post-harvest process for the generation of good quality chocolate. This process is catalyzed by environmental microbes that colonize the cocoa pulp in a predictable microbial succession. Here we present a robust study of the population dynamics of the microbial cocoa fermentation using high-throughput sequencing methods that allow us to evaluate the inter- and intra-specific diversity of the microbial community, the dominance and transitivity of strains (within and between regions), their interactions. 

Methods: Three cocoa farms were selected for the study, each representing an important cacao producing region in Colombia. Sampling was conducted during two different periods in 2016. During each period cocoa beans were collected during the whole fermentation process, with a 12 hour from the upper and intermediate zones of wooden fermenters. A total 126 cocoa bean samples were collected. DNA extraction and library preparation was done for 16S rRNA genes and ITS region. All libraries were sequenced with the V2 kit using a MiSeq platform (Illumina). Results: Sequencing of 16s rRNA and ITS libraries generate a total of 3’560.120 paired reads. The taxonomic classification of reads for all samples showed a reduced diversity at the 97% OTUs level. The microbial community composition at the 97% OTU-level was similar in all fermentation systems (department independent) and showed the expected microbial succession, were microbial abundance is dominated by enteric bacteria then transition to Lactic Acid Bacteria (LAB) and finally to Acid Acetic Bacteria(AAB). The analysis of oligotyping divided each OTU, into finer groups (oligotypes groups), for Yeast, Enterobacteria, LAB and AAB groups, 12, 6, 11 and 19 olygotypes were found respectively. Interestingly the dominant strains seem to be the same on all evaluated farms. While transient strains appeared only at the transition of the microbial succession suggesting more abundant and diverse resources. 

Conclusions: In conclusion, these results show that there is a larger that previously reported microbial diversity in the Cocoa fermentation process and highlights the importance of fine level diversity tools to monitor the dominance and resilience of microbial populations. Such approaches are necessary for the improvement of fermentation protocols and the development and validation of starter cultures.
Session Number: 500
Session Type: Rapid Fire
Session Number: 500
Session Type: Rapid Fire
Session Title: The Wonderful World of Agricultural and Environmental Microbiomes
Session Start Date Time: 6/10/2018 4:30:00 PM
Session End Date Time: 6/10/2018 5:15:00 PM
Session Primary Track: Applied and Environmental Science
Abstract Control Number: 9133
Poster Board Number:

Abstract Title:
Moderator
Primary Author Block:
Edward Dudley; Pennsylvania State Univ., University Park, PA
Abstract Body:
Characterizing the Effect of Different Organic Cover Crop Techniques on Microbial Diversity

Primary Author Block:
L. Wu, C. Wang, D. Parson, S. Fankhauser; Oxford Coll. of Emory Univ., Oxford, GA

Abstract Body:
Background and Methods: The microbial community within a piece soil is essential to the fertility of soil and success of agricultural crops, yet a substantial portion of microbial life within the soil remains uncultivated and under-explored. Many species of bacteria can play mutualistic as well as harmful roles in the life cycle of a crop, and specific practices can play a deciding factor in generating different microbial communities. One such strategy, cover cropping, involves planting a specific species of plant and then mowing down to the ground (without harvesting) for restoring and recycling nutrients in the soil and developing beneficial biota. To understand how various cover cropping techniques affect soil microbial communities, we used next-generation sequencing to compare the bacterial presence under a single-, multi-species, as well as a lack of cover crop influence. An experimental plot was established with the three different cover crop treatments in triplicate. After six weeks of cover crop treatments, a broccoli crop was planted. Soil samples were taken at key points throughout the experiment. From each soil sample, specific nutrient levels were determined. Additionally, DNA was isolated and Illumina sequencing was performed using universal primers specific for the V3 and V4 regions of the 16s rDNA gene. Sequence analysis is ongoing, but we are comparing the presence of known mutualistic and parasitic microbial genera.

Results and Conclusions: Data thus far indicate that the cover crop techniques correlate with differential impact on soil fertility and crop-outcome. An overall 6-7% increase in active carbon content in single- and mega-mix treatments, respectively, as well as an overall 10% decrease in the control group were observed. On average, plants from the control group exhibited the greatest mass but also the least length while the mega-mix group demonstrated the lowest mass but the greatest length. The mega-mix group demonstrated a 6% increase in average leaf area while the single-mix exhibited a 6% decrease when compared to the control group. As the experiment is on-going, the microbial data, carbon and nitrogen content measurements, and final broccoli assessment are yet to be interpreted. These results will be the first to systematically investigate how cover crops alter microbial communities of the soil. While our study is specific to an agricultural setting, our results will contribute to the growing body of knowledge of how specific plant communities can change the microbial ecology of the soil.